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On the Effect of heparin, sodium polyanethol sulfonate (Liquoid Roche) and tribasic sodium citrate on human leukocytes in vitro[1)]

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Since the discovery of heparin in 1918 by Howell, a number of investigations on the effect of this coagulation-inhibiting substance on isolated cells and tissues have been published. These studies comprise tests on the effect of heparin on a) mesenchymal cells, b) bacteria and c) leukocytes.

On mesenchymal cells of the chicken, A. Fischer (6) observed inhibited growth by heparin. Zakrzewsky (19) reported on the same effect. With a certain dosage, heparin can thus represent a different substance for growing mesenchymal cells.

Direct effects of heparin on bacteria could be observed so far neighter in an inhibiting nor in an enhancing sense. According to Mahorner and Ochsner (13) the growth of staphilococci is not influenced by Hirudin or heparin.

The following is known about the effect of heparin on isolated white blood cells: Fischer (5) found that heparin is bound on the cell surface by intact living leukocytes. Buser (2) reports that the leukocytes lose their adhesion under the influence of heparin. Jorpes (9) points out in a monography on heparin that if heparitized blood is used, the white elements must be counted within two hours, otherwise they show a tendency to agglutination, and they may even disappear. Grimberg, Chamreff and Pellier (7), use in their comparison between heparin and citrate as a preservative the resistance of the peroxidase reaction as an expression of the cell activity; they indicate that the peroxidase reaction remains longer positive in heparin blood than in citrate blood. The effect of heparin on the white blood cells can also be observed in tests on the entire organism. Thus Jucker (10) found shortly

after intravenous injection of heparin in the rabbit and in man a drop of leukocytes with subsequent leukocytosis. He assumed a similar mechanism of action as it was described for the first time by Staub (17) for the leukocyte drop after intravenous injection of other macromolecules, for example, of glycogen. What we found so far in investigations of the effect of heparin on white blood cells concerns thus properties which are not directly related to the defense mechanism proper of the leukocytes.

Since heparin has gained considerable clinical importance in the last years and it is frequently given in transfusions (Schuerch (16) and in the prophylaxis and thereapy of thrombosis (Lenghenhager (12)) to patients suffering from infections, it seemed to us of interest to test its influence on the cellular defense mechanism of the human blood [2]. As a measure of the defense activity we selected the migration, as well as the phagocytosis in vitro. At the same time we extended our investigations to two different coagulation-inhibiting substances, namely sodium polyanthol sulfonate (Liquoid Roche) and tribasic sodium citrate.

Technique

Migration of leukocytes: We used principally the leukocyte film method suggested by Carrel and worked out by Kiaer (11) for chicken blood. Quantitative tests of the mobility of mammal leukocytes with the bottle method have hardly been carried out so far to our knowledge, Only in R. Meier (14) do we find a reference that this has been tried. The results of the respective study, however, are based likewise on chicken leukocytes. The contractibility of the human plasma makes the production of uniform leukocyte film pieces as a starting material impossible. We therefore formed the film of human leukocytes in the chicken plasma, then placed the film pieces in the autological plasma and serum. We obtain thus an abundant and uniform migration.

In practice we proceed as follows: Puncturing of the arm vein with a winged needle, then gently defibrinating the blood by keeping itin constant rotary movement. A small compact clot is formed, the number of leukocytes

and the morphological picture remain unchanged with a proper technique. Centrifuging the defibrinated blood for 10 minutes at 4000 rpm, draining the serum, cooling the cell residue, and adding about 2 cc ice-cold chicken-plasma into a centrifuge tube of 10 cc. Shaking and centrifuging again for 10-15 minutes in ice-cooled centrifuge beakers at 4000 rpm. In this time a very compact leukocyte film is formed, and the added chicken plasma coagulates toward the end of the centrifuging. It can then be removed from the tube as a clear cylinder. The leukocyte film sticks to its under surface. It is separated and permits cutting of about 20 leukocyte pieces of 2 mm side length. The edge zones should not be used. In order to obtain a good leukocyte film, the blood should contain about 15,000 cells. We therefore took the blood for the tests in acute states of inflammation. The leukocyte pieces thus obtained are placed in Carell bottles in the following medium: 0.4 cc citrate plasma of the same individual (contains 0.35% citrate); 0.2 cc serum of the same individual; 1.0 cc tyrode, which contains 10% chicken embryo extract; 1 drop of 5% $CaCl_2$. After coagulation of the medium, we wait for 30 minutes and then overlay the substance to be tested in Ringer solution. The nature of the highly effective coagulation-inhibiting substances made the desirable addition to the defibrinated blood impossible; tests made in this respect yielded to irregular leukocyte films. The indicated concentrations are thus only approximate values, since an amount of liquid corresponding to the solid phase of the double contraction was overlaid, assuming a uniform diffusion. Naturally the entire process must take place under sterile precautions. Each substance was first tested 3-4 times with 4 bottles each, which had been subjected to the same treatment. The series was completed with the general test represented in fig. 3 with 2 Carrel bottles per concentration. The pictures were obtained by projection; Fig. 4 shows the coefficients migration surface/starting piece. They were obtained by planimetering the projection pictures. The coefficient of the control cultures is assumed with 100, the error margin being $\pm$ 15%.

Phagacytosis Since the coagulation-inhibiting substances themselves were the object of our investigation, we proceeded as follows with a modified Wright method (18): gentle defibrination as described above(with a low leukocyte number, enrichment of the leukocytes, if necessary). Mixing of the following elements: 1 cc blood (enriched with leukocytes, if necessary); 1.25 cc of the liquid to be tested: 0.25 cc bacterial suspension (24 hour Coli bouillon culture is washed three times in Ringer and suspended in half the amount of Ringer). According to previous experiences, this solution should contain about 2.10^9 germs/cc (Allgoewer (1)). This mixture is shaken for 15 minutes in the incubator at 37 degC, then blood smears are made and stained according to May-Gruenwald-Giemsa. Then the Coli bacilli phagocyitzed in 100 neutrophils are counted. The graphic reproduction of the result is in % of the germs phagocytized in the controls.

Concentrations used: We wanted to test the doses used in the clinic or in animal experiments and multiples thereof - but for the citrate a narrow limit was set by the necessity of isotonia, so that coagulation-effecting concentrations were also tested with this substance. The clinically used concentration range for heparin and the citrate is reproduced in the figures.

Results

Migration: A leukocyte film piece placed in the incubator shows after one hour a migration zone of about 1 mm width. If we follow this spread with the fastmotion film, as it was done in previous tests, we can clearly recognize the active movement of the individual leukocytes. The initial velocity of the leukocytes is about 1 mm/h, disregarding the fact that the movements of the individual leukocytes are not straight. The migrations have attained their maximum after 8-14 hours. Their maximum radius in our tests was 5 mm (fig.1). The qualitative structure of the migration zone will be discussed in another connection. Nevertheless it can be said that in the various cultures examined in this respect, the various elements always showed the same behavior. In the periphery we find the polynuclear

leukocytes mixed with the generally more centrally located monocytes. Next to the starting piece wesee the eosinophils and basophils arranged rather close together, together with the lymphocytes. This corresponds to the facts known so far about the mobility of the various white cells. Whether the migration of the various cells has been hindered or enhanced by the substances used has not been investigated.

Fig. 1 14-hour leukocyte culture in Carrel bottle

Fig.2 Neutrophilic leukocyte with 5 phagocytized Coli bacilli

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Fig. 3 Migration areas after 14 hours

hatched; concentration in the transfusion blood

c ross-hatched: calculated concentration in the total blood after i.v. injection

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The picture obtained by the projection of the test cultures (fig.3) of two cultures each, which were obtained under the same conditions, show sufficient regularity so that a quantitative evaluation of the result is possible. The use of the quotient of the surface areas migration zone/ starting piece as a measure of the leukocyte migration seems to us permissible under the given conditions. The essential feature of this method is that a linear phenomenon, like the leukocyte migration, is expressed by an area quotient. This type of representation is also justitied because the same number of leukocytes were present in each starting piece per unit of area, and because the "unmigrated area" represents a good expression for the resultant of the innumerable individual movements.

Phagocytosis: The influence of the various substances can be seen from fig.4. Fig. 2 shows a neutrophil with 5 germs. The average number of phagocytized germs per neutrophil was 4.2 in the controls of the test reproduced in fig.4.

If we put the two effects side by side, we see the following:

Fig.4

- - - migration (plotted the quotients mig ation/area/starting piece)

____ phagocytosis in % of the controls

o heparin

triangle: liquoid

+ citrate

hatched: concentrations in the transfusion blood

cross-hatched; calculated concentration in the total blood after i.v. injection

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Heparin leaves the migrations of the leukocytes to a great extent unaffected. In most cases a clear inhibition starts only at a concentration of 40 mg%. In some tests the inhibition of the migration was less pronounced, but it still existed. In two cases we found a slight increase of the migration under heparin, but these were blood samples of older test persons whose leukocyte cultures in the control tests showed little migration. In contrast to the migration capacity, the phagocytosis is inhibited by heparin at much lower concentrations. Up to 5 mg%, that is, in the clinically used doses, it remains very close to the error margin of the method of determination. With a geometrically progressive dose series, the number of phagocytized germs drops slowly. Liquoid presents another picture.

The migration is already considerably inhibited at 10 mg%. The phagocytosis drops practically to zero within a narrow concentration range. There seems to be here not only a quantitative, but also a qualitative difference in action with regard to heparin.

With sodium citrate the difference of action on phagocytosis and migration is most pronounced. While at low citrate concentrations a very great inhibition of the phagocytosis is observed, one would be tempted to speak with the same dosage of an increase of the migration capacity of the leukocytes. In clinically used citrate concentrations, however, the migration also proves to be inhibited. If hypertonic citrate solution is used, that is, if citrate solutions of over 3.55% are added to the blood, the inhibiting effect is considerably increased.

Discussion

The tests described here show that heparin has the least harmful effect on the migration capacity and phagocytosis of the human leukocytes, compared to sodium polyanethol sulfonate (liquoid) and sodium citrate.

No reliable data are available on the mechanism of inhibition of higher concentrations of heparin on the migration capacity and phagocytosis of the leukocytes. We could ask ourselves whether a similar mechanism is involved here as it was described by Zakrzewsky (19) for the action of heparin on mesenchymal cells. He assumes the inhibition of a "stimulator," which he identifies as prothrombin. The low inhibiting effect of heparin on the migration capacity and phagocytosis of the white blood cells of man can not account for the leukocyte drop after heparin injection observed by Jucker (10). The doses used by Jucker are far below the inhibiting concentrations in vitro. The phenomenon of the leukocyte drop after intravenous injection of high-molecular substances thus seems to be caused by extracellular factors. The view by Staub (7) that the leukocyte drop represents a passive problem- the large elements get stuck in the pulmonary capillaries- received indirectly further support. Rigdon and Wilson (15) found neither the capillary permeability nor the development of localized inflammatory reactions nor the bacterial phagocytosis in the focus of inflammation changed in rats after heparin doses. But we can not readily compare the results of these authors with our own results because the heparin concentration obtained in the blood by Rigdon and Wilson is not indicated. Besides, we only estimated the degree of phagocytosis.

Unlike heparin, liquoid considerably inhibits the migration capacity of the leukocytes, even close to concentrations which are required to inhibit coagulation. The phagocytosis is inhibited even more. The drop of the phagocytosis curve is most pronounced with liquoid among the tested substances. Since the anticimplement effect of liquoid is known (van der Hoeden (8)), and Delaway (3) has demonstrated the necessity of the complement for

the migration and phagocytosis, the liquoid inhibition of the migration and phagocytosis seems understandable. But whether these findings also apply to hyman leukocytes is an open question. If van de Hoeden doubts it, since he found that the non-specific phagocytosis is only slightly reduced under the action of liquoid in patients suffering from brucellosis, we can not verify it for the leukocytes of healthy individuals with regard to Coli.

That citrate inhibits the phagocytosis of leukocytes was already found in 1909 by Wright (18). Possibly we are dealing here with a calcium deficiency effect. Because, if citrate plasma is recalcified before it comes in contact with the leukocytes no inhibition of the migration capacity of human leukocytes is found according to our orientative tests made so far. Delaunay (3) found in guinea pig leukocytes which had been placed in citrate for a short time, reversibility of the inhibition by the addition of calcium. Investigations of the reversibility of the citrate effect in dependence of the dose and time of action (transfusion blood !) and the significance of the amounts of transfused citrate are not yet completed.

Finally it can be said that the phagocytosis takes place much faster under the given conditions than the migration, and that the influence on both properties is not the same at each moment. This suggests a certain independence of the two phenomena.

Summary

1. An attempt is made to determine quantitatively the migration capacity of isolated human leukocytes.

2. It is found that a characteristic migration area of the leukocytes can be obtained for a certain individual at a certain time.

3. An examination of the various coagulation-inhibiting substances according to this method and according to the phagocytosis test shows the following result:

Heparin: Little influence on the migration (as a rule inhibition only at concentrations over 40 mg%), gradual inhibition of phagocytosis with geome-

trically progressive doses. Clinically used doses have hardly any inhibiting effect on the two functions.

Liquoid: Rather great inhibiting effect on migration,total inhibition of phagocytosis within a narrow concentration range.

Sodium citrate: In low concentration, migration and phagocytosis are differently affected: hardly detectable increase of migration with great inhibition of phagocytosis. Both functions, however, are inhibited with clinically used doses.

4. In the examination of the above mentioned coagulation-inhibiting substances, the inhibition of the migration and phagocytosis did not always go hand in hand. This suggests a certain independence of the two phenomena.

References

Footnotes:

(1) A donation made by Sandoz A.G. for the construction of the Basler Buergerspital permitted the Surgical University Clinic to equip its laboratories with the necessary scientific apparatus; a part of these laboratories comprises two rooms for the artificial growing of tissues, where particularly the behavior and reactions of the human tissues are to be observed. The report by Martin Allgoewers, the first from this laboratory is an expression of gratitude of the Clinic to the donor and to Prof. Dr. A. Stell who was instrumental in getting this donation.

(2) As a heparin preparation we used liquenim, made available to us by the courtesy of Hoffmann-LaRoche.

(Translated by Carl Demrick Associates,Inc/IE/t)

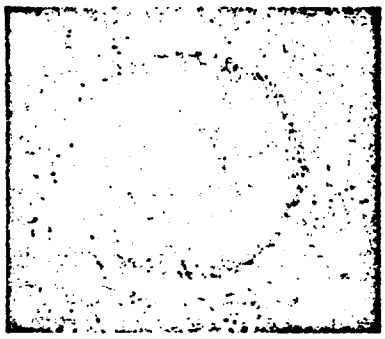

Figure 1

Figure 2

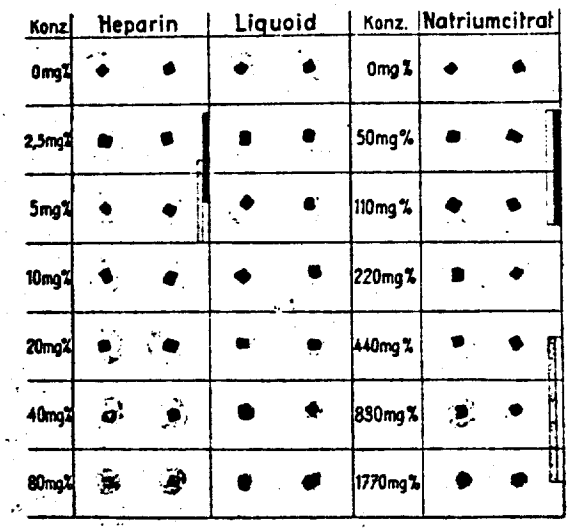

Figure 3

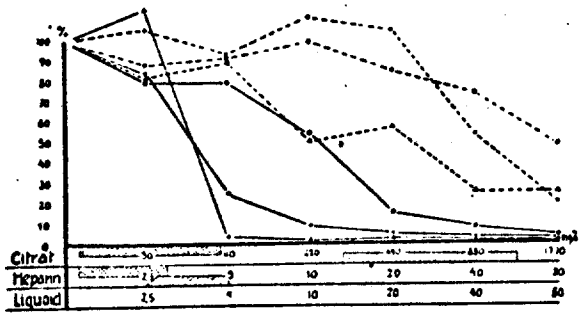

Figure 4

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Citrat 2

7. Das, was den „insuffizienten" Herzmuskel charakterisiert, ist die Notwendigkeit, schon in der Ruhe unter erhöhter Ausgangsspannung = Mehrdehnung zu arbeiten. Dem liegt offenbar ein Nachlassen, eine Unvollkommenheit der Elastizität oder des Tonus des Herzmuskels infolge Ueberdehnung zugrunde. Die segensreiche Wirkung der Herzmittel, speziell der Digitaliskörper, die in toxischen Gaben systolischen Herzstillstand hervorrufen, besteht in einer Tonisierung und Verkürzung der gedehnten Muskelfaser, so daß die notwendige Ausgangsspannung unter geringer Dehnung erreicht wird. Die „Wiederherstellung der Reservekraft" besteht darin, daß nunmehr nach Verkleinerung der erweiterten Herzhöhle und Tonisierung des Muskels wieder eine Steigerung der Ausgangsspannung *ohne* Ueberdehnung und damit wieder eine Leistungssteigerung ermöglicht wird.

In der medizinischen Jugend unter *Riegel* wurde nur mit Digitalis per os, meist mit dem Infus behandelt, und wenn das nicht genügte, wurden Southeysche Röhrchen gelegt oder Calomel gegeben. Eine heroische, oft unerhört erfolgreiche, manchmal auch recht fatale Therapie. So riesige Diuresen wie nach der alten Calomelmedikation habe ich später nicht wieder gesehen (aber auch nicht mehr so üble Nebenwirkungen wie dysenterieartige Enteritis und schwerste Stomatitis) bei Anwendung der neuen Hg-Präparate, die wir den „Bayer"-Werken verdanken. Und die Magenstörungen nach Digitalis waren oft recht unerfreulich.

In der Diagnostik gab es noch keine Röntgenbilder des Herzens, geschweige ein Ekg., und erst *Riegels* Nachfolger *Moritz* brachte uns die Orthodiagraphie bei und versuchte die Ergebnisse der Herzperkussion der Röntgensilhouette anzupassen. Damals war viel von der Tastperkussion die Rede, und auch ich bildete mir fest ein, bei der Perkussion entsprechende Tasteindrücke zu haben. Aber *Moritz* überzeugte uns, daß bei ganz fest geschlossenen Ohren überall, auch im Bereich des vollen Lungenschalles, absolute Dämpfung herrscht. Die Tastperkussion erwies sich als Selbsttäuschung.

In Dortmund (1905—1908) haben wir schon gelegentlich Digitalis intravenös gegeben. Durch meine Berufung nach Mannheim kam ich mit *Albert Fränkel* in Berührung und dadurch kam es, daß wir schon damals Strophanthin i.v. als Regel anwandten, und wir waren von den Erfolgen begeistert. Bei dem Versuch, das Strophanthin durch die Tinctura Strophanthi zu ersetzen, erlebte ich, daß 20 Tropfen und mehr per os ohne deutliche Wirkung blieben, aber bei intravenöser Anwendung von 1—2 Tropfen der Tinktur trat eine eklatante Wirkung ein, einmal sogar ein atrioventrikulärer Block. Dem entspricht die Feststellung von *Krötz* über die Unwirksamkeit der rektalen Strophanthinanwendung.

Die Erolge der i.v. Strophanthinbehandlung übertrafen alles, was ich von der Digitalis bei *Riegel* gesehen hatte. War die „Kompensation" erreicht, so gingen wir zur Dauerbehandlung mit kleinen Digitalisdosen über, unterstützt durch möglichst flüssigkeitsarme Ernährung, die dann späterhin so salzfrei als möglich gestaltet wurde. Bei den Patienten, die es fertig brachten, sie streng durchzuführen, war der Dauererfolg so eklatant, daß später Herzmittel nicht mehr nötig wurden.

In Halle (1918—1927) habe ich auch sofort die Strophanthinbehandlung der Herzinsuffizienz, die damals an manchen Kliniken noch verboten war, eingeführt und mich wieder von den die orale Digitalisbehandlung weit übertreffenden Erfolgen überzeugt, aber auch von der ausgezeichneten Wirkung der salzlosen Trockenkost nicht nur bei Nierenkranken und Hypertonikern, sondern gerade auch bei den Kranken mit Neigung zu feuchter Herzinsuffizienz. Einen großen Fortschritt bedeutete aber auch die Lieferung gut verträglicher Hg-Präparate durch die pharmazeutische Industrie, mit denen man die Kranken nicht nur entwässern, sondern auch entsalzen und durch periodische Anwendung vor Rückfällen der Wassersucht bewahren konnte. Der einzige Nachteil der Strophanthinbehandlung war der Zwang zur intravenösen Injektion, und nicht jeder Mitarbeiter war gleich geschickt, diese in manchen Fällen bei schlechten Venen doch recht schwierige Methode zu beherrschen. Und paravenöse Injektionen sind sehr schmerzhaft, die intramuskulären von Myokombin keineswegs schmerzlos und in der Wirkung unsicher.

Hier haben die klassischen Arbeiten von *Stoll* einen großen Fortschritt gebracht. Seine Reindarstellung der genuinen Lanataglykoside hat einen ganz neuen Boden für die Digitalistherapie geschaffen und die Nachteile der alten peroralen Digitalismedikation wie die der intravenösen Strophanthintherapie überwunden. Schon die Reindarstellung kristallisierter Präparate ist ein großer Gewinn; sie ermöglicht endlich ihre Dosierung nach Gewicht an Stelle der biologischen Testung nach Toxizität, sie sichert die Haltbarkeit und verbessert durch Wegfall der Ballaststoffe die Verträglichkeit. Vor allem aber ermöglicht sie die intravenöse und, was uns als das Wichtigste erscheint, auch ihre *intramuskuläre* Anwendung. Das ist von großer Bedeutung für die Praxis geworden. Fräulein *v. Sachs* hat über unsere ausgezeichneten Erfolge mit Digilanid berichtet. Es wurde peroral überraschend gut vertragen; bei intravenöser Anwendung steht es dem Strophanthin nicht nach; die intramuskuläre Injektion ist schmerzlos und dabei ebenso wirksam wie die intravenöse. Das gleiche gilt von dem nur in der Digitalis lanata enthaltenen Digilanid C oder Cedilanid. Diese Präparate eigenen sich auch ganz besonders für die sogenannte Erhaltungs- oder Dauerbehandlung, die wir evtl. viele Monate, ja Jahre durchführen mit dem Rate, sicherheitshalber am Wochenende 2 Tage mit der Medikation auszusetzen. In Fällen schwerster portaler Stauung versagt begreiflicherweise die perorale Medikation, da ist die intravenöse oder intramuskuläre Injektion notwendig. Letztere war bemerkenswerter Weise in einzelnen Fällen selbst dann noch wirksam, wenn die i.v. Strophanthinbehandlung versagt hatte.

Sarre und Mitarbeiter fanden unter der Digitaliswirkung eine Verkürzung der pathologisch verlängerten Anspannungszeit und eine Verlängerung der Austreibungszeit. Diese interessante Wirkung auf die Dynamik des insuffizienten Herzens trat bei peroraler Verabreichung von Cedilanid erstaunlich rasch, schon nach einer halben Stunde ein, um in 1½ Stunden ihr Maximum zu erreichen. Mit der gleichen Methode haben die Autoren die Wirkung der i.v. Injektion von 0,2 mg Strophanthin und 0,6 mg Digilanid untersucht und einen sehr ähnlichen Kurvenverlauf festgestellt.

Ueber die Dosierung hat *Krötz* sich maßgebend geäußert. Hinzuzufügen wäre nur noch, daß nach unseren klinischen Erfahrungen und den Experimenten *Sarres* Digilanid i.v. bei Koronarinsuffizienz zu vermeiden und Cedilanid per os nur in sehr kleinen Dosen von 0,1—0,2 mg anzuraten ist. Hier scheint Strophanthin bzw. Strophosid — wegen geringerer Vaguswirkung? — den Vorzug zu verdienen. Aber im übrigen sind wir dank der Forschungen von *Stoll* heute nicht mehr auf dieses Präparat allein angewiesen und der Kranke nicht mehr auf einen Arzt, der gut i.v. spritzen kann. Kurz, seine Darstellung der reinen kristallinischen Präparate der Herzmittel bedeutet einen so großen Fortschritt, daß wir ihm nicht dankbar genug sein können.

Blut

Aus der chirurgischen Universitätsklinik Basel
Vorsteher: Prof. Dr. *C. Henschen*

Ueber die Wirkung von Heparin, polyanetholsulfosaurem Natrium (Liquoid Roche) und tribasischem Natriumcitrat auf menschliche Leukozyten in vitro[1])

Von Martin Allgöwer, Assistent der Klinik

Seit der 1918 erfolgten Entdeckung des Heparins durch *Howell* sind eine Reihe von Untersuchungen über die Wirkung dieser gerinnungshemmenden Substanz auf isolierte Zellen und

[1]) Eine von der Firma Sandoz A.G. für den Neubau des Basler Bürgerspitals gemachte Schenkung ermöglichte der chirurgischen Universitätsklinik die Ausstattung ihrer Laboratorien mit der nötigen wissenschaftlichen Apparatur; ein Teil dieser Laboratorien umfaßt zwei Räume für künstliche Züchtung von Geweben, wobei vor allem Verhalten und Reaktionsweisen der menschlichen Gewebe verfolgt werden sollen. Die Mitteilung *Martin Allgöwers*, erste aus diesem Laboratorium, sei Ausdruck des Dankes der Klinik an die Stifterin und an Herrn Prof. Dr. *A. Stoll* als den Vermittler der Donation. *C. Henschen.*

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Gewebe bekannt geworden. Diese Arbeiten umfassen Versuche über den Effekt von Heparin auf a) mesenchymale Zellen, b) Bakterien und c) Leukozyten.

An mesenchymalen Zellen des Huhns hat *A. Fischer* (6) eine Wachstumshemmung durch Heparin beobachtet. Ueber die gleiche Wirkung berichtet *Zakrzewsky* (19). Bei bestimmter Dosierung kann somit Heparin für wachsende mesenchymale Zellen einen differenten Stoff darstellen.

An Bakterien konnten bis jetzt direkte Wirkungen des Heparins weder in hemmendem noch in förderndem Sinne festgestellt werden. Nach *Mahorner* und *Ochsner* (13) wird beispielsweise das Staphylokokkenwachstum durch Hirudin oder Heparin nicht beeinflußt.

Ueber die Wirkung von Heparin auf isolierte weiße Blutzellen ist folgendes bekannt: *Fischer* (5) stellt fest, daß das Heparin auf der Zelloberfläche von intakten, lebenden Leukozyten gebunden wird. *Buser* (2) teilt mit, daß die Leukozyten unter Heparineinwirkung die Haftfähigkeit verlieren. *Jorpes* (9) weist in seiner Monographie über Heparin daraufhin, daß bei Verwendung von heparinisiertem Blut die Zählung der weißen Elemente innerhalb zwei Stunden zu erfolgen habe, da sie sonst Tendenz zur Agglutination und sogar zum Verschwinden zeigen. *Grimberg, Chamraeff* und *Pellier* (7) nehmen in ihrem Vergleich zwischen Heparin und Citrat als Konservierungsmittel die Persistenz der Peroxydasereaktion als Ausdruck der Zellvitalität; sie geben an, daß im Heparinblut die Peroxydasereaktion länger positiv bleibt als im Citratblut. Die Wirkung des Heparins auf die weißen Blutelemente kann auch in Versuchen am Gesamtorganismus geprüft werden. So hat *Jucker* (10) kurz nach der intravenösen Verabreichung von Heparin beim Kaninchen und beim Menschen einen Leukozytensturz mit nachheriger Leukozytose feststellen können. Er vermutete einen ähnlichen Wirkungsmechanismus, wie ihn *Staub* (17) für den Leukozytensturz nach intravenöser Injektion von anderen Makromolekülen, z. B. von Glykogen, erstmals beschrieben hat. Was uns bis jetzt an Untersuchungen über Heparinwirkung auf weiße Blutzellen zugänglich war, betrifft somit Eigenschaften, die mit den eigentlichen Abwehrfunktionen der Leukozyten in keinem direkten Zusammenhang stehen.

Nachdem Heparin in den letzten Jahren zu beträchtlicher klinischer Bedeutung gelangt ist, und es bei Transfusionen (*Schürch* [16]) und zur Thromboseprophylaxe und -therapie (*Lenggenhager* [12]) oft infektionsgefährdeter Patienten gegeben wird, schien es von Interesse, seinen Einfluß auf den zellulären Abwehrapparat des menschlichen Blutes zu prüfen[2]). Als Maß der Abwehraktivität wählten wir die Wanderung sowie die Phagozytose in vitro. Gleichzeitig dehnten wir unsere Untersuchungen auf zwei verschiedenartige, gerinnungshemmende Stoffe aus, nämlich polyanetholsulfosaures Natrium (Liquoid Roche) und tribasisches Natriumcitrat.

Technik

Leukozytenwanderung: Im Prinzip verwendeten wir die von *Carrel* angeregte und von *Kizer* (11) für Hühnerblut ausgearbeitete Leukozytenfilmmethode. Quantitative Untersuchungen über die Beweglichkeit von Säugetierleukozyten mit der Flaschenmethode sind unseres Wissens bis jetzt kaum ausgeführt. Lediglich bei *R. Meier* (14) finden wir einen Hinweis, daß dies versucht worden sei. Die Resultate der betreffenden Arbeit stützen sich jedoch ebenfalls auf Hühnerleukozyten. Die Kontraktilität des menschlichen Plasmas verunmöglicht das Herstellen von gleichmäßigen Leukozytenfilmstückchen als Ausgangsmaterial. Wir bilden deshalb den Film menschlicher Leukozyten im Hühnerplasma, setzen dann die Filmstückchen im autologen Plasma und Serum an. So erhalten wir eine reichliche und gleichmäßige Auswanderung.

Praktisch gehen wir wie folgt vor: Punktieren der Armvene mittelst Flügelnadel, schonendes Defibrinieren, indem man das Blut in dauernder rotierender Bewegung hält. Es bildet sich ein kleines kompaktes Gerinnsel, wobei die Leukozytenzahl und das morphologische Bild bei richtiger Technik unverändert bleiben. Zentrifugieren des defibrinierten Blutes 10 Minuten bei 4000 Umdrehungen, Absaugen des Serums, Kühlen des Zellrückstandes und Zufügen von ca. 2 ccm eisgekühltem Hühnerplasma in ein Zentrifugenröhrchen von 10 ccm. Aufschütteln und erneutes Zentrifugieren während 10—15 Minuten in eisgekühlten Zentrifugenbechern bei 4000 T. In dieser Zeit bildet sich ein sehr kompakter Leukozytenfilm und das zugefügte Hühnerplasma gerinnt gegen Ende des Zentrifugierens. Es kann als klarer Zylinder dem Röhrchen entnommen werden. An seiner Unterfläche haftet der Leukozytenfilm. Dieser wird abgetrennt und erlaubt das Schneiden von ca. 20 Leukozytenstückchen von 2 mm Kantenlänge. Die Randpartien sollen nicht verwendet werden. Zur Erzielung eines guten Leukozytenfilms sollte das Blut ca. 15000 Zellen/cmm enthalten. Wir haben deshalb das Blut für diese Versuche bei akuten Entzündungszuständen entnommen. Die so erhaltenen Leukozytenstückchen werden in Carrelflaschen angesetzt in folgendem Medium: 0,4 ccm Citratplasma des gleichen Individuums (enthält Citrat 0,35%); 0,2 ccm Serum des gleichen Individuums; 1,0 ccm Tyrode, die 10% Hühnerembryonalextrakt enthält; 1 Tropfen 5% $CaCl_2$. Nach dem Gerinnen des Mediums wird 30 Minuten zugewartet und dann die auszutestierende Substanz in Ringerlösung überschichtet. Die Natur der hochwirksamen gerinnungshemmenden Stoffe verunmöglichte die an sich wünschenswerte Zugabe zum defibrinierten Blut — in dieser Richtung unternommene Versuche ergaben zu ungleichmäßige Leukozytenfilme. Die angegebenen Konzentrationen geben also nur einen ungefähren Anhaltspunkt, indem bei Annahme einer gleichmäßigen Diffusion eine der festen Phase entsprechende Flüssigkeitsmenge von doppelter Konzentration überschichtet wurde. — Selbstverständlich muß der ganze Arbeitsprozeß unter sterilen Kautelen vor sich gehen. Jede Substanz wurde vorerst einzeln 3—10mal mit je 4 gleichartig behandelten Fläschchen geprüft. Den Schluß bildete der wiedergegebene Uebersichtsversuch (Abb. 3) mit 2 Carrelflaschen pro Konzentration. Die Bilder sind durch Projektion gewonnen. In Abb. 4 sind die Koeffizienten Auswanderungsfläche/Ausgangsstück aufgezeichnet. Sie wurden durch Planimetrieren der Projektionsbilder erhalten. Der Koeffizient der Kontrollkulturen ist gleich 100 gesetzt — die Fehlergrenze liegt bei ±15%.

Phagozytose: Da die gerinnungshemmenden Substanzen selbst das Ziel unserer Prüfung waren, gingen wir in Modifikation der *Wright*schen Methodik (18) folgendermaßen vor: schonendes Defibrinieren wie oben beschrieben. (Bei niederer Leukozytenzahl allenfalls Anreichern der Leukozyten.) Mischen folgender Elemente: 1 ccm Blut (allenfalls an Leukozyten angereichert); 1,25 ccm zu untersuchende Flüssigkeit; 0,25 ccm Bakteriensuspension (24stündige Colibouillonkultur wird dreimal in Ringer gewaschen und in der halben Ringermenge suspendiert). Nach früherer Erfahrung dürfte diese Suspension ca. 2.10^9 Keime/ccm enthalten (*Allgöwer* [1]). Diese Mischung wird 15 Minuten im Brutschrank bei 37° geschüttelt, hernach werden Blutausstriche angefertigt und nach May-Grünwald-Giemsa gefärbt. Dann zählt man die in 100 Neutrophilen phagozytierten Colibazillen. Die kurvenmäßige Wiedergabe des Resultates erfolgt in Prozent der in den Kontrollen phagozytierten Keime.

Verwendete Konzentrationen: Es sollten die in der Klinik oder im Tierversuch verwendeten Dosierungen und ihre Mehrfachen geprüft werden — beim Citrat war aber durch das Gebot der Isotonie eine enge Grenze gesetzt, so daß bei dieser Substanz auch gerinnungsunwirksame Konzentrationen zur Prüfung gelangten. Der klinisch verwendete Konzentrationsbereich ist für Heparin und für das Citrat in den Abbildungen wiedergegeben.

Ergebnisse

Wanderung: Ein in den Brutschrank verbrachtes Leukozytenfilmstück zeigt nach einer Stunde einen nahezu millimeterbreiten Auswanderungssaum. Verfolgt man diese Ausbreitung im Zeitrafferfilm, wie wir dies in früheren Versuchen getan haben, so läßt sich die aktive Bewegung der einzelnen Leukozyten deutlich erkennen. Die anfängliche Geschwindigkeit der Leukozyten beträgt gegen 1 mm/Stunde, wobei nicht berücksichtigt ist, daß die Bewegungen der einzelnen Leukozyten nicht geradlinig verlaufen. Die Wanderungszone hat nach 8—14 Stunden ihr Maximum erreicht. Ihr Radius betrug in unseren Versuchen maximal 5 mm (Abb. 1). Ueber den qualitativen Aufbau der Auswanderungszone soll in einem anderen Zusammenhang berichtet werden. Immerhin kann festgestellt werden, daß sich in den daraufhin untersuchten Kulturen die verschiedenen Elemente stets gleichartig verhielten. In der Peripherie finden sich die Polynukleären, vermischt mit den im

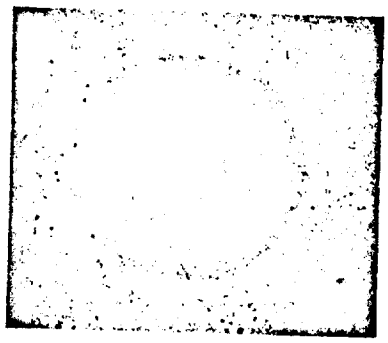

Abb. 1. 14stündige Leukozytenkultur in Carrelflasche.

[2]) Als Heparinpräparat stellte uns die Firma F. Hoffmann-LaRoche freundlicherweise Liquemin zur Verfügung.

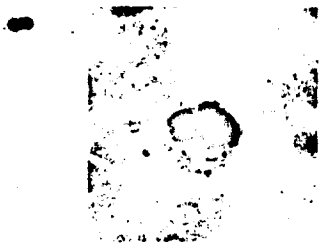

Abb. 2. Neutrophiler Leukozyt mit 5 phagozytierten Colibazillen.

allgemeinen mehr zentralwärts gelegenen Monozyten. In der Nähe des Ausgangsstückes sieht man die Eosinophilen und Basophilen ziemlich dicht angeordnet, zusammen mit den Lymphozyten. Dies entspricht den bis jetzt bekannten Tatsachen über die Beweglichkeit der einzelnen weißen Elemente. Ob durch die verwendeten Substanzen einzelne Elemente in bezug auf die Wanderung selektiv behindert oder gefördert wurden, ist nicht untersucht worden.

Die durch Projektion der Versuchskulturen erhaltenen Bilder (Abb. 3) von je zwei unter denselben Bedingungen stehenden Kulturen lassen eine genügende Gleichmäßigkeit erkennen, so daß die quantitative Auswertung der Resultate möglich ist. Die Benutzung des Quotienten der Flächeninhalte Wanderungszone/Ausgangsstück als Maß der Leukozytenwanderung scheint uns unter den gegebenen Bedingungen gestattet. Das Wesentliche an dieser Methode ist, daß ein linear verlaufendes Phänomen wie die Leukozytenwanderung durch einen Flächenquotienten ausgedrückt wird. Eine solche Darstellungsart rechtfertigt sich auch deshalb, weil in jedem Ausgangsstück pro Flächeneinheit gleichviel Leukozyten vorhanden waren, und weil außerdem die „bewanderte Fläche" einen guten Ausdruck für die Resultante der unzähligen Einzelbewegungen darstellt.

Phagozytose: Der Einfluß der verschiedenen Substanzen ist aus Abb. 4 zu ersehen. Abb. 2 zeigt einen Neutrophilen mit 5 Keimen. — Die durchschnittliche Zahl der phagozytierten Keime pro Neutrophilen betrug in den Kontrollen des in Abb. 4 wiedergegebenen Versuches 4,2.

Stellt man die beiden Wirkungen nebeneinander, so ergibt sich das folgende Bild:

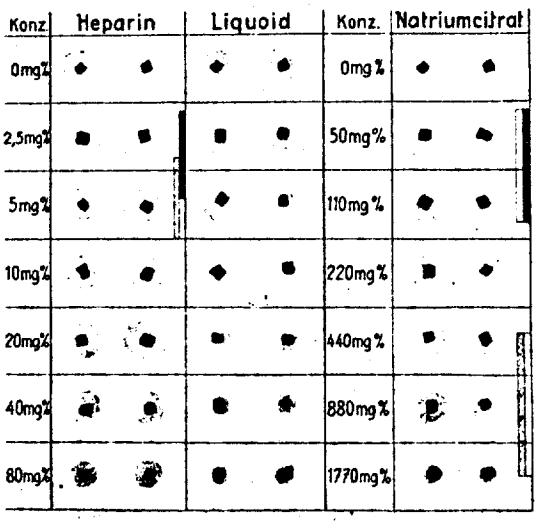

Abb. 3. Auswanderungsflächen nach 14 Stunden.

Konzentration im Transfusionsblut.

Berechnete Konzentration im Gesamtblut nach intravenöser Gabe.

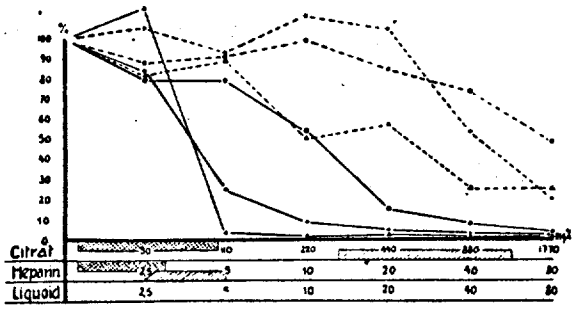

Abb. 4.

Auswanderung (Aufgetragen die Quotienten Auswanderungsfläche/Ausgangsstück).

Phagozytose in Prozent der Kontrollen.

o Heparin.

▲ Liquoid.

– Citrat.

Konzentrationen im Transfusionsblut.

Berechnete Konzentration im Gesamtblut nach intravenöser Gabe.

Heparin läßt die Leukozytenwanderung weitgehend unbeeinflußt. In den meisten Fällen beginnt eine deutliche Hemmung erst bei einer Konzentration von 40 mg%. In einigen Versuchen war die Wanderungshemmung bei dieser Konzentration zwar weniger stark, aber immer noch vorhanden. In zwei Fällen konnten wir eine leichte Steigerung der Auswanderung unter Heparin feststellen. Jedoch handelte es sich um Blutproben älterer Versuchspersonen, deren Leukozytenkulturen im Kontrollversuch eine geringe Auswanderung zeigten. Im Gegensatz zu der Wanderungsfähigkeit wird die Phagozytose durch Heparin in viel niederer Konzentration gehemmt. Bis zu 5 mg%, d. h. in den klinisch verwendeten Dosen, bleibt sie sehr nahe an der Fehlergrenze der Bestimmungsmethode. Bei geometrisch progressiver Dosenfolge sinkt die Zahl der phagozytierten Keime langsam ab.

Liquoid bietet ein anderes Bild. Die Migration ist schon bei 10 mg% deutlich gehemmt. Die Phagozytose fällt innerhalb eines engen Konzentrationsbereiches praktisch auf null. Es scheint hier nicht nur ein quantitativer, sondern auch ein qualitativer Wirkungsunterschied gegenüber Heparin vorhanden zu sein.

Beim *Natriumcitrat* ist die Verschiedenheit der Wirkung auf Phagozytose und Wanderung am deutlichsten. Während bei niederen Citratkonzentrationen eine sehr starke Hemmung der Phagozytose zu verzeichnen ist, wäre man versucht, bei gleicher Dosierung von einer Steigerung der Wanderungsfähigkeit der Leukozyten zu sprechen. In klinisch verwendeten Citratkonzentrationen erweist sich dann allerdings auch die Wanderung als gehemmt. Wird hypertonische Citratlösung benützt, d. h. wird das Blut mit Citratlösungen von über 3,55% versetzt, so verstärkt sich der hemmende Effekt beträchtlich.

Diskussion

Die hier mitgeteilten Versuche zeigen, daß Heparin im Vergleich zu polyanetholsulfosaurem Natrium (Liquoid) und Natriumcitrat auf die Wanderungsfähigkeit und Phagozytose der menschlichen Leukozyten den geringsten schädigenden Effekt besitzt.

Ueber den Hemmungsmechanismus höherer Konzentrationen von Heparin auf Wanderungsfähigkeit und Phagozytose der Leukozyten ist nichts Sicheres bekannt. Man könnte sich fragen, ob ein ähnlicher Mechanismus im Spiele ist, wie ihn *Zakrzewsky* (19) für die Wirkung des Heparins auf mesenchymale Zellen beschrieben hat. Er glaubt an die Hemmung eines „Stimulators", den er als Prothrombin identifiziert. Die geringe Hemmwirkung des Heparins auf Wanderungsfähigkeit und Phagozytose der weißen Blutkörperchen des Menschen vermag den von *Jukker* (10) beobachteten Leukozytensturz nach Heparininjektion nicht zu erklären. Die von *Jucker* verwendeten Dosen liegen weit unter den in vitro hemmenden Konzentrationen. Somit erscheint

das Phänomen des Leukozytensturzes nach intravenöser Injektion von hochmolekularen Substanzen extrazellulär bedingt. Die Ansicht von *Staub* (17), daß der Leukozytensturz ein passives Phänomen — Hängenbleiben der großen Elemente in den Lungenkapillaren — darstelle, erhält indirekt eine weitere Stütze. *Rigdon* und *Wilson* (15) haben bei Ratten nach Heparingaben weder die Kapillarpermeabilität noch die Entwicklung lokalisierter entzündlicher Reaktionen noch die Bakterienphagozytose im Entzündungsherd verändert gefunden. Die Resultate dieser Autoren können wir nicht ohne weiteres mit unseren Ergebnissen vergleichen, weil die im Blut erreichte Heparinkonzentration von *Rigdon* und *Wilson* nicht angegeben wird. Außerdem haben sie den Grad der Phagozytose nur schätzungsweise ermittelt.

Im Gegensatz zu Heparin hemmt Liquoid die Wanderungsfähigkeit der Leukozyten in ausgesprochener Weise, und zwar schon nahe bei Konzentrationen, die zur Gerinnungshemmung benötigt werden. Noch stärker wird die Phagozytose gehemmt. Der Abfall der Phagozytosekurve ist unter den geprüften Substanzen beim Liquoid am deutlichsten. Nachdem die Antikomplementwirkung des Liquoids bekannt ist (*van der Hoeden* [8]), und von *Delaunay* (3) die Notwendigkeit des Komplements für die Wanderung und Phagozytose nachgewiesen wurde, scheint die Liquoidhemmung der Migration und Phagozytose erklärlich zu sein. Ob diese Befunde auch für menschliche Leukozyten Gültigkeit haben, bleibt dahingestellt. Wenn *van der Hoeden* dies bezweifelt, indem er feststellt, daß bei Brucellosekranken die unspezifische Phagozytose nur eine geringe Einbuße unter Liquoideinwirkung erleidet, so können wir das für die Leukozyten gesunder Individuen gegenüber Coli nicht bestätigen.

Daß Citrat die Phagozytose der Leukozyten hemmt, hat schon *Wright* (18) im Jahre 1909 festgestellt. Möglicherweise handelt es sich um eine Calcium-Mangelwirkung. Wenn man nämlich Citratplasma vor dem Kontakt mit den Leukozyten rekalzifiziert, so ist nach unseren bisherigen orientierenden Versuchen keine Hemmung der Wanderungsfähigkeit menschlicher Leukozyten festzustellen. *Delaunay* (3) hat für Meerschweinchen-Leukozyten, die kurze Zeit in Citrat gelegen haben, Reversibilität der Hemmwirkung durch Calciumzugabe festgestellt. Wir haben dies bis jetzt für die Phagozytose menschlicher Leukozyten nicht bestätigen können. Untersuchungen über die Reversibilität der Citratwirkung in Abhängigkeit von Dosis und Zeit der Einwirkung (Transfusionsblut!) und die Bedeutung der transfundierten Citratmengen sind noch nicht beendet.

Abschließend läßt sich feststellen, daß unter den gegebenen Bedingungen die Phagozytose durchwegs schneller beeinträchtigt wird als die Migration, und daß die Beeinflussung beider Eigenschaften nicht in jedem Moment gleichsinnig erfolgt. Dies läßt auf eine gewisse Unabhängigkeit der beiden Phänomene schließen.

Zusammenfassung. 1. Es wird der Versuch unternommen, die Migrationsfähigkeit isolierter menschlicher Leukozyten quantitativ zu erfassen.

2. Es zeigt sich, daß für ein bestimmtes Individuum zu einer bestimmten Zeit eine charakteristische Auswanderungsfläche der Leukozyten erhältlich ist.

3. Die Prüfung verschiedener gerinnungshemmender Substanzen nach dieser Methode und nach dem Phagozytosetest ergibt folgendes:

Heparin: Geringer Einfluß auf die Migration (in der Regel Hemmung erst in Konzentrationen über 40 mg%), allmähliche Hemmung der Phagozytose bei geometrisch progressiver Dosenfolge. Klinisch verwendete Dosen wirken auf beide Funktionen kaum hemmend.

Liquoid: Ziemlich starker Hemmeffekt auf die Migration, totale Hemmung der Phagozytose innerhalb eines engen Konzentrationsbereiches.

Natriumcitrat: In niederen Konzentrationen werden Phagozytose und Migration verschieden beeinflußt: kaum nachweisbare Steigerung der Migration bei starker Hemmung der Phagozytose. Beide Funktionen werden aber in den klinisch verwendeten Dosierungen gehemmt.

4. Bei Prüfung der angeführten gerinnungshemmenden Substanzen gingen die Hemmung der Migration und der Phagozytose nicht immer parallel. Dies läßt auf eine gewisse Unabhängigkeit der beiden Phänomene schließen.

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La protidogenesi ematica nella dottrina e nella pratica medica

Di D. Cesa Bianchi

Le nostre conoscenze sulla emoprotidogenesi si può dire che stiano finalmente uscendo dallo stato di nebulosità in cui si trovavano sino a pochi anni or sono, grazie ai progressi che si sono conseguiti con la ricerca sperimentale e sopratutto con le osservazioni comparate, biochimica ed anatomoclinica, condotte su una ormai sufficientemente estesa casistica. Può essere quindi utile riassumere le conclusioni di maggior importanza pratica, derivanti dal lavoro compiuto in questo campo, da più di un decennio, nella Clinica medica dell'Ateneo milanese.

Va innanzitutto osservato che in opposizione alla vecchia concezione, ancora oggi tradizionalmente ripetuta nei trattati, che attribuiva la formazione delle sieroproteine al fegato, attualmente sembra invece accertato che in condizioni fisiologiche essa venga effettuata essenzialmente dal midollo osseo. A quest'organo pertanto spettano funzioni emopoietiche nel senso più lato della parola e, cioè, nel senso di fornire al circolo non solo le cellule ematiche, ma anche il substrato non organizzato strutturalmente, nel quale esse sono immerse: il cosidetto plasma, che rappresenta l'interstizio di quel tessuto *sui generis* che è il sangue. Anche nella vita post-natale si manterrebbe quindi quella stretta correlazione ontogenetica che esiste nell'embrione, a livello degli isolotti di Wolff, fra la formazione della parte liquida e della parte corpuscolata del sangue.

Questa concezione deriva da un complesso di ricerche comparative fra il quadro protidemico midollare e periferico, alle quali fanno riscontro, in campo clinico, le osservazioni di gravi ipoprotidemie unicamente giustificate da alterazioni del midollo osseo.

Così, ad esempio, in un caso di *Jürgens* si è osservato una completa scomparsa del fibrinogeno ematico in seguito alla invasione pressochè totalitaria del midollo osseo da parte di metastasi di blastoma prostatico. Così in un caso di *Villa*, di una grave emopatia decorsa per 16 anni, nella quale in coincidenza con l'instaurarsi della atrofia mieloide si stabilì una sindrome anasarcatica. Così, probabilmente, in un caso di *Oettel* e sopratutto in un caso di recente osservato nella mia clinica sul quale dovremo ritornare brevemente più avanti.

Queste nuove conoscenze sulla sede di formazione delle plasmaproteine, hanno già avuto anche una applicazione pratica, in quanto ci permettono di interpretare il meccanismo d'azione di un recente metodo di cura del diabete insipido, che appariva sinora piuttosto misterioso, e precisamente l'irradiazione roentgen delle epifisi ossee, introdotta nella pratica ed in parecchi casi applicata con successo evidente, in seguito ad una fortunata osservazione di *Cignolini*. Con tale procedimento si viene difatti a influenzare direttamente la protidogenesi, con conseguenti modificazioni della

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THE EFFECTS OF ADDITIVES ON RADIATION-RESISTANCE OF Cl. botulinum IN MEAT

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Abstract

THE EFFECTS OF ADDITIVES ON RADIATION-RESISTANCE OF Cl. Botulinum IN MEAT.
Beef ground round inoculated with 1 million spores of Cl. botulinum per gram and containing various additives was exposed to gamma radiation. Mild heat treatment applied after irradiation reduced the survival of Cl. botulinum, strain A-5 spores in raw ground beef. Heat applied before or during irradiation appears to have no effect. Sodium chloride, calcium chloride, mustard oil (allyl isothiocyanate), nutmeg, sodium citrate and Versene reduced the dose or irradiation required to inactivate Cl. botulinum spores (strains A-5 and B-115) in raw ground beef. Spores were inactivated in samples (irradiated at 2.0, 2.5 and 3.0 Mrad) which contained sodium nitrate (1000 ppm) plus sodium chloride (2.5%). Similar results were obtained when sodium nitrite (200 ppm) was substituted for sodium nitrate, except that there was evidence of spore survival in 1 of 120 cans irradiated at 2.0 Mrad. Spore destruction was based upon the absence of spores and mouse-lethal toxin in meat sub-cultures made from cans incubated at 35°C for 120 days. Spores were not destroyed when exposed to 2.5 or 3.0 Mrad in the absence of sodium nitrate, sodium nitrite or sodium chloride. Furthermore, the use of these chemicals individually together with radiation was ineffective. The additives alone in the absence of radiation also did not cause spore destruction. Radiation levels of 2.0, 2.5 and 3.0 Mrad, when used with sodium chloride at 1.5 or 2.0% and sodium nitrate at 500 ppm or sodium nitrite at 100 ppm, were ineffective.

INTRODUCTION

Cl. botulinum is one of the most radiation resistant food intoxication sporeformers [1-3]. This fact has created a serious problem in the radiation sterilization of non-acid foods. It is the objective of food scientists to make radiation sterilized foods equal in safety, from the public health standpoint, that achieved for other accepted methods for commercially processed foods. At the radiation level now recommended for sterilization, serious defects in odour, flavour, colour, and texture occur which make the product unacceptable.

Recent investigations indicate that the radiation level required to destroy Cl. botulinum may be reduced appreciably by the use of common food additives, or to some degree by post-radiation heat treatment. Spices and chemicals of various kinds as well as mild heat treatments are used in many types of specialty meat products. Chemical additives, such as sodium chloride, sodium nitrite, and sodium nitrate, have wide use as preservatives in meat [4]. Their effect on Cl. botulinum was demonstrated by Yesair and Cameron [5], who found a 70% reduction in spore counts brought about by the use of 0.1% sodium nitrate, 0.005% sodium nitrite, or 2% sodium chloride in a meat infusion agar. Bulman and Ayres [6] also noted that nitrite inhibited spore germination of a putrefactive anaerobe (P.A. 3679) in inoculated meat emulsions. These

chemical additives have been more recently suggested for canned meat and other foods [7].

The present study was undertaken to determine the effect of spices, heat and common chemical additives on the dose of gamma radiation required to destroy spores of Cl. botulinum inoculated into canned ground steak. Spores of type 33-A, 5A, and 115B were used, since recent studies [8] showed these to be among the most radiation-resistant.

MATERIALS AND METHODS

Preparation of spores

Original cultures of Cl. botulinum type A5, A33, and B115 were obtained from the National Canners Association, Washington, D.C. and the Quartermaster Food and Container Institute for the Armed Forces, Natick, Mass. The large number of spores necessary for these experiments were obtained by introducing a 0.5% inoculum, heat shocked for 10 minutes at 80°C [9], into freshly steamed trypticase-thioglycollate broth (trypticase, 5%; Bacto-peptone, 1.5%; glucose, 0.01%; sodium thioglycollate, 0.1%; pH 7.0). The inoculated flasks were then incubated at 37°C for 7-8 days, to allow maximum spore formation.

After the growth period spores were harvested by centrifugation, washed twice with M/15 phosphate buffer (pH 7.0), combined, and stored in phosphate buffer solution (titre 1×10^8 spores/ml) at 4°C until needed. Before use, viable spore titres were checked by inoculating trypticase-thioglycollate broth with appropriate heat shocked dilutions of the spore suspension.

Radiation source

All cans were irradiated at the Gamma Test Facilities, Arco, Idaho. Dosimetry was checked every eight hours by ceric sulphate bottles or ion chamber dosimeters. In the case of the submersible water bath the dosimeters were located inside the cans at the top, bottom, and centre inside the inner chamber.

Meat packs

The meat was high quality (85% lean and 15% fat and fibre) fresh ground round obtained from a locally operated, government inspected, meat packing plant. 1000-g portions were inoculated with heat-shocked spores to a concentration of approximately 10^6 spores per gram. 100 g of inoculated meat was packed into each baby food can (202 × 204). The cans were sealed using an automatic can sealer, rinsed in 1000 ppm chlorinated detergent solution, packed in wet ice, and shipped to the Gamma Test Facilities where they were held at wet ice temperature until irradiated.

Heat source

The concurrent heat treatment was provided by specially designed equipment which could be lowered into the irradiation field. Hot water, kept at a constant temperature (± 0.5°C) was provided from two 25-gallon reservoirs. The hot-water supply also served as the come-up tank and the water supply reservoir for the temperature-controlled submersible bath. A pump was used to circulate the water through the submersible bath and back to the reservoir.

The temperature in the submersible bath was determined experimentally by a thermocouple in the inner chamber. A temperature drop of approximately 5°C was recorded between the hot-water reservoir and the tank when exposed under water at 20°C in the irradiation field. Control experiments were made prior to the actual irradiation tests under simulated conditions as described below.

The come-up times for the meat packs were determined on an automatic recorder by inserting thermocouples in the centre of cans [10] filled with meat. The cans were immersed in water 5°C higher than the desired temperature. It was observed experimentally that 35 minutes was the maximum time required for a can to reach the desired internal temperature irrespective of the final heat level.

The cans treated before irradiation were heated before being shipped to the irradiation source. Cans treated after irradiation were heated upon their return from the source. During transporting and/or storage the cans were held at wet-ice temperature.

Spice and chemical additives

Meat was weighed out in 1000-g quantities and the spices and/or chemicals added in the quantities indicated in the tables. These were usually added in amounts permitted in foods under the existing regulation of the Food and Drug Administration [11]. The meat mixture was held at 4°C for 16 h to allow the additives to diffuse thoroughly throughout the meat.

The test sample was then inoculated with 10^6 heat-shocked spores per gram, and thoroughly mixed. 100 g of the test samples was packed into each baby food can (202 × 204), sealed, packed in insulated wet-ice containers, and shipped to the Gamma Test Facility for irradiation.

Assay for spore survival

The irradiated samples returned to Oregon State University were incubated at 37°C and observed for a hard swell indicative of spoilage. Most spoilage, if it were to occur, would take place within a week to 10 days.

Those cans which did not spoil after a time lapse of 30 to 120 days were aseptically opened. Five grams of meat were removed from the centre of the sample and from various parts of the can for a total of 15 g and placed into a screw-cap test tube. Seven millimetres of freshly steamed trypticase-thioglycollate broth was added per tube and incubated for a minimum of 48 h at 37°C.

The incubated sub-cultures were centrifuged at 800 × g for 20 min. The supernatant fluid was decanted off and divided into two portions.

One portion was transferred to a sterile culture tube (15×150 mm), and served as the active preparation; the remaining portion was transferred into another culture tube and boiled for 15 min to inactivate any toxin present, centrifuged, and used as a control. Both sets of culture tubes were covered with rubber-hooded stoppers and taped shut.

0.2 ml of a sample was then injected into a 15-20 g white mouse intraperitoneally. Two mice received the unboiled inoculum and one received the boiled inoculum. Toxicity of the preparation was characterized by the typical symptoms of botulinum toxin poisoning followed by death. The mice were observed for toxin effects for a period of 3 days. Toxic symptoms were absent in the boiled control.

Wagenaar and Dack [12] and Bonventre and Kempe [13] observed that the amount of radiation needed to inactivate the toxin was considerably more than that needed for sterilization. Hence, if the toxin was present, it could be introduced with the original inoculum and could

TABLE I. VISIBLE SPOILAGE AND MOUSE LETHALITY OF GROUND ROUND INOCULATED WITH Cl. botulinum (A-5), (10^6 spores/g)

Irradiation level (Mrad)	Time heat applied	Treatment temperature (°C)									
		20	60	65	70	75	77.5	80	82.5	85	87.5
0.0		5[a]	5	5	5	5	5	5	5	5	5
	before	5	5	5	5	5	4	5	2	5	2
2.0	during	5	5	5	5	4	5	4	4	3	3
	after	5	5	5	5	3	5	1	5	1	1
	before	1	4	1	1	0	0	5	0	3	2
2.5	during	1	5	1	2	0	2	2	1	0	2
	after	1	1	0	1	0	0	1	1	0	1
	before	0	0	1	1	2	1	5	0	5	1
3.0	during	0	0	1	1	0	1	0	0	0	0
	after	0	0	0	0	1	0	0	0	0	1

Figures underlined indicate no visible spoilage due to can swelling; however Cl. botulinum was recovered and shown to be toxic.

[a] Five replications per sample (500 cans per experiment).

remain after radiation processing. Thus, to ascertain the presence of viable spores in the toxin-containing samples, heat-shocked sub-cultures were made from the 15-g meat samples. These sub-cultures were then examined microscopically for the presence of spores and toxin-producing ability.

RESULTS AND DISCUSSION

Results shown in Table I indicate that heat, if used in the radiation sterilization process, should be applied following, not before or during, the irradiation treatment. Heat applied before or during the irradiation actually increased the radiation resistance of the type A spores, especially in the temperature range 80-87.5°C. This effect might be explained in one or more of the following ways:

(a) Shrinkage and loss of water due to the effect of the heat on the meat substrate could reduce the diffusion of the lethal ions produced by the irradiation

(b) Fats rendered by the heat treatments might afford protection by coating the spore's surface simulating dehydration and thus increase their resistance to the irradiation.

(c) Active sulphur groups produced by heating the protein of the meat may act as free radical acceptors affording further protection to the spores.

Again referring to Table I, it appears that heat treatments in a number of instances affected the spores in such a way that germination did not occur during incubation of the cans. However, upon opening the cans and sub-culturing the contents, spore germination and toxin production could be demonstrated. This phenomena was especially pronounced in the heating before and during irradiation and occasionally in the heat treatment following irradiation. Such a practice could create a potentially dangerous condition. Before heat treatments can be recommended for reducing radiation sterilization doses, further confirmatory experiments should be carried out.

Sodium chloride increased the lethal effect of radiation on the spores of Cl. botulinum. Experimental data shown in Table II indicate that the lethal action of sodium chloride was exerted most effectively at a concentration of 3.0% at an irradiation level of 3.0 Mrad. Lower levels of sodium chloride had little or no demonstrable effect. Strain A-5 was less resistant than strain B-115 to the combined effects of salt and irradiation. Calcium chloride, at a concentration of 3.0%, was as effective as an equivalent concentration of sodium chloride in destroying the spores of strain B-115. The chloride ion may be the active agent in enhancing the lethal action of radiation since NaCl and $CaCl_2$ at near equivalent concentrations were equally effective.

A number of spices were investigated including nutmeg, sage, coriander, cinnamon, allspice, caraway, cardamon, mace, marjoram, celery, thyme, garlic, red pepper, mustard and cloves. Of these only mustard appeared synergistic with irradiation. Nutmeg, coriander and sage exhibited a slight effect.

Data in Table III show that mustard oil (allyl isothiocyanate), at a concentration 0.1%, increased the lethal action of irradiation with the

TABLE II. MOUSE LETHALITY OF IRRADIATED GROUND ROUND (10^6 spores/g)

Mrad	No. of samples[a]	Uninoculated control	Inoculated control No. salt (a)	Inoculated control No. salt (b)	Sodium chloride 1% (a)	Sodium chloride 1% (b)	Sodium chloride 2% (a)	Sodium chloride 2% (b)	Sodium chloride 3% (a)	Sodium chloride 3% (b)	Sodium chloride 4% (a)	Sodium chloride 4% (b)
0.0	2	0	18	18	18	18	18	18	18	18	18	18
2.0	2	0	18	18	18	18	6	18	0	18	0	0
2.5	2	0	18	18	16	18	16	18	0	18	0	0
3.0	2	0	18	18	1	9	1	10	0	2	0	0

[a] Nine replications per sample, 792 cans per experiment.

(a) A-5; (b) B-115

TABLE III. MOUSE LETHALITY OF INOCULATED GROUND ROUND (10^6 SPORES/g)

Irradiation level (Mrad)	Uninoculated control	Inoculated control (a)	Inoculated control (b)	Mustard 0.1% (a)	Mustard 0.1% (b)	Mustard 0.1% + 1% NaCl (b)	Mustard 0.1% 2% NaCl (b)	Mustard 0.1% 3% NaCl (b)
0.0	0	9[a]	9	9	9	9	9	9
1.5	0	9	9	9	9	9	9	9
2.0	0	9	9	0	0	0	0	0
2.5	0	9	9	0	0	0	0	0
3.0	0	9	9	0	0	0	0	0

[a] 1 sample, 9 replications (360 cans per experiment).

(a) A-5; (b) B-115

TABLE IV. MOUSE LETHALITY OF GROUND ROUND PLUS SODIUM CITRATE OR VERSENE WITH Cl. botulinum (TYPE B115), (10^6 SPORES/g)

Level of irradiated (Mrad)	No. of samples	Uninoculated control	Inoculated control	Sodium citrate 0.01 %	Sodium citrate 0.1 %	Sodium citrate 1.0 %	Versene (di-Na) salt 0.186	Versene-CA (food grade) 0.05%	Versene-CA (food grade) 0.5%
0.0	2	0	18	18	18	18	18	18	18
2.5	2	0	18	18	18	9	0	18	18
3.0	3	0	27	4	0	0	0	0	1

Number of cans from which spores of Cl. botulinum were recovered and on sub-culturing were toxic for mice.

9 replications per sample (504 cans per experiment).

total inactivation of spores occurring at 2.0 Mrad. Again, strain B-115 appeared more resistant than strain A-5. Sodium chloride and mustard oil in combination was no more effective than mustard alone.

The results with the chelating agents, ethylene-diamine-tetra-acetic acid (EDTA) and sodium citrate, are shown in Table IV. Concentrations of 0.186% Versene and 0.05% Versene CA, in inoculated samples irradiated at 3.0 Mrad, inactivated spores of Cl. botilinum. However, 0.5% Versene

CA appeared to enhance survival. A concentration of 0.1% sodium citrate was sufficient to inactivate spores at 3.0 Mrad. It is possible that both EDTA and sodium citrate may induce by their chelating properties the pregermination state, a condition in which the spores are more sensitive to irradiation.

Sodium citrate also exhibited a desirable effect on the organoleptic qualities of raw irradiated beef. The texture was preserved and there appeared to be a marked reduction of the odours characteristic of irradiated meat.

TABLE V. SPOILAGE OF IRRADIATED CANNED GROUND ROUND STEAK INOCULATED WITH Cl. botulinum SPORES (33-A) PLUS SODIUM NITRATE OR SODIUM NITRITE PLUS SODIUM CHLORIDE

Additive	Radiation dose (Mrad)	Total cans	Swollen cans[a]	Unswollen cans containing toxin[b]	Unswollen cans and no toxin
None	0	25	25	-	-
	2.0	25	25	-	-
	2.5	25	23	0	2
	3.0	25	15	0	10
Sodium nitrate (1000 ppm) plus 2.5% sodium chloride	0	25	25	-	-
	2.0	125	0	0	125
	2.5	25	0	0	25
	3.0	25	0	0	25
Sodium nitrite (200 ppm) plus 2.5% sodium chloride	0	25	25	-	-
	2.0	125	1	5	119
	2.5	25	0	0	25
	3.0	25	0	0	25

Inoculum consisted of 10^6 spores per g of meat.

[a] Cans were held at 35°C until hard swells developed. All cans which showed hard swells were examined microscopically for spore development and for lethal concentrations of toxin.

[b] Cans not having hard swells within 120 days were sub-cultured and tested for toxin by mouse assay and examined microscopically for outgrowth of spores.

TABLE VI. SPOILAGE OF IRRADIATED GROUND ROUND STEAK MIXED WITH VARIOUS ADDITIVES AND INOCULATED WITH Cl. botulinum SPORES (33-A)

Additive	Radiation dose (Mrad)	Total cans	Swollen cans[a]	Unswollen cans containing toxin[b]	Unswollen cans and no toxin
None (1000 ppm)	0	25	25	-	-
	2.0	25	5	20	-
	2.5	25	10	15	0
	3.0	25	1	24	0
Sodium nitrate (200 ppm)	0	25	25	-	-
	2.0	25	12	13	0
	2.5	25	13	12	0
	3.0	25	4	21	0
Sodium chloride (2.5%)	0	10	10	-	-
	2.0	25	15	10	0

Inoculum consisted of 10^6 spores per g of meat.

[a] Cans were held at 35°C until hard swells developed. All cans which showed hard swells were examined microscopically for spore development and for lethal concentrations of toxin.

[b] Cans not having hard swells within 120 days were sub-cultured and tested for toxin by mouse assay and examined microscopically for outgrowth of spores.

Preliminary experiments revealed that the use of gamma irradiation in combination with sodium chloride (1.5 or 2.0%) plus sodium nitrite (100 ppm) or sodium nitrate (500 ppm) was not effective in causing the destruction of Cl. botulinum spores which had been inoculated into canned ground round steak. Hence, the chemical additives in these experiments were used at higher concentrations but still within the maximal tolerable levels as specified by the Food and Drug Administration [11].

The results obtained when various dosages of gamma irradiation were used in combination with sodium chloride (2.5%) and sodium nitrate (1000 ppm) are shown in Table V. The use of these additives with any

of the radiation dosages was effective with 25 cans, an additional 100 cans were tested to increase the number of samples.

The results obtained when sodium nitrite (200 ppm) was substituted for sodium nitrate in the preceding experiment were similar (Table V). Under these conditions, 1 can of 125 developed a hard swell within the 120-d incubation period, and 5 of 124 samples contained sufficient toxin to cause lethal effects when injected into mice. Hence, on the basis of these results and at the levels tested, sodium nitrite at 200 ppm does not appear to be as effective as sodium nitrate at 1000 ppm when used with gamma irradiation to inactivate Cl. botulinum spores.

The data also reveal that the use of either the combined additives or irradiation alone (2.0, 2.5 and 3.0 Mrad) was not effective in inactivating the spores or in preventing the development of hard swells. Microscopic examination of the irradiated samples (no additives) with hard swells revealed rods morphologically similar to Cl. botulinum, which indicated the presence of Cl. botulinum in these samples.

On the basis of the results (Table VI), the use of the various additives individually is not as effective as a combination of additives. When 2.0 Mrad of irradiation were used in combination with these additives, it was once again observed that sodium nitrate at 1000 ppm and sodium chloride alone are less effective than either sodium nitrite or sodium nitrate.

Toxin may be present in cans exhibiting no swelling or visible deterioration, in agreement with Kempe and Graikoski [14] who suggested that Cl. botulinum spores may be inactivated by irradiation but still be capable of germination and growth through one or two vegetative cycles. This would explain the presence of demonstrable toxin in meat showing no gross spoilage. However, it has been our experience that where toxin was present there were also viable spores in cans showing no signs of spoilage. Hence, it appears that the presence of lethal concentrations of toxin was associated with spore germination and subsequent cell growth rather than with toxin introduced with the original inoculum.

The observation that combinations of either sodium nitrate or sodium nitrite with sodium chloride lower the dose of gamma radiation necessary to destroy Cl. botulinum offers the possibility of the use of these additives in radiation-preserved foods. Additional studies directed toward the practicability of using these additives in food sterilization and pasteurization programmes are suggested.

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Infrared absorption study of metal oxides in the low frequency region (700–240 cm^{-1})

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Abstract—The characteristic frequencies of oxides of 52 metals have been studied in the region 700–240 cm^{-1}. Data for oxides of metals of different valence states and the frequencies of polymorphic forms of several oxides are presented. A particle size of 10 μ or smaller was found to give the best representative spectrum.

INTRODUCTION

IN THE past several years the majority of metal oxide compounds have become important for various reasons. Two of the more important of these are their high temperature stability for use as refractory bodies, and their optical properties for use as infrared lenses. The metal oxides have not been extensively studied in the infrared region, and an investigation of the absorption characteristics of these materials should therefore be of interest.

A review article on inorganic materials by LAWSON [1] revealed very little information on oxides, and the papers by MILLER [2, 3] and co-workers deal primarily with the spectra of a large number of inorganic salts. Some Raman data have been presented on diverse oxides [4] consisting of the salts of molybdates, tungstates, arsenates and chromates. The earlier infrared studies in the rock salt region deal primarily with individual compounds, with papers on magnesia [5], titania [6] and yttria [7]. Some spectra of oxides have extended into the low frequency region; and there are papers on oxides of beryllium [8], silicon [9], aluminum [10], boron [11], arsenic [12, 13], tellurium [14], titanium and

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vanadium [15]. Several papers by Parodi [16, 17, 18] report frequencies on a number of divalent metal oxides.

In a fundamental study of the properties of refractory materials it was found that the infrared spectrum, in the region between 700 and 240 cm^{-1}, was more informative for these oxides than the conventional rock salt region. For this reason the study of the absorption characteristics of a large number of metal oxides was undertaken.

Experimental

The instrument used was the Perkin–Elmer Model 102, a spectrophotometer designed and constructed specifically for our Laboratory. The instrument has a prism-grating double beam monochromator and records linearly in wavenumbers. In the automatic mode the spectrophotometer is capable of recording spectra from 4000–240 wavenumbers. For the conditions under which the spectra reported in this paper were obtained, a KBr and CsI foreprism were used in combination with a 30 μ blazed grating to cover the region 700–240 cm^{-1}, with a spectral slit width of 4 cm^{-1}.

The majority of the samples were commercial chemicals of C.P. grade while the rare-earth oxides were of spectrographic purity. Several of the polymorphic forms were prepared in our laboratory by standard procedures. The crystal form of each oxide was checked by X-ray diffraction.

All of the spectra were obtained as Nujol mulls. Nujol has only a weak absorption band at 720 cm^{-1} and proves to be an ideal mulling agent for this region. Compounds that gave indications of having a band under the 720 Nujol band were re-run as KBr disks. The Nujol mull samples were dispersed between CsI plates. The instrument was flushed with dry air until satisfactory energy checks were obtained at 250 cm^{-1}.

Results and Discussion

A discussion of each infrared spectrum of the metal oxides would exceed the space limitations of this paper. Only a general discussion concerning particle size, rare-earth oxides, polymorphism and a few of the individual compounds will be presented.

Table 1 is an alphabetical listing of all the compounds that have been studied. The second column shows the compound or compounds that result from different valence states and polymorphic forms. The third column shows the positions of the bands. An asterisk after the compound formula means that the bands in the spectrum for that particular compound are poorly defined. The relative intensities of the characteristic bands are included in this Table; however, they refer only to bands within each individual spectrum. This information has no meaning, from one curve to another, when dealing with these solid inorganic substances in Nujol mulls. The band shapes are noted where they are broad or occur as a shoulder on other bands. Where the crystal structure of a compound has not been noted the common stable form was used.

[15] S. M. Ariya and M. V. Golomolzina, *Soviet Phys.—Solid State* **4**, 2142 (1963).
[16] M. Parodi, *Compt. rend.* **204**, 1111 (1937).
[17] M. Parodi, *Compt. rend.* **204**, 1636 (1937).
[18] M. Parodi, *Compt. rend.* **205**, 906 (1937).

Table 1. Characteristic frequencies of metal oxide infrared absorption bands

Metal	Formula	Region (cm^{-1})
Aluminum	αAl_2O_3	575 s, 432 s, 375 sh
Antimony	Sb_2O_3 (cubic)	740 s, 685 sh, 590 m, 550 sh, 482 m, 383 s, 345 w, 322 w, 265 s
Arsenic	As_2O_3 cubic	500 sh, 473 s, 355 sh, 340 s
Barium	BaO	503 sh, 483 s, 305 sh, 283 m, 255 m
	BaO_2	(No bands 700–250)
Beryllium	BeO	(No bands 700–250)
Bismuth	αBi_2O_3	645 w, 505 s, 345 s
Boron	B_2O_3	765 sb, 638 m, 543 m
Cadmium	CdO	(No bands 700–250)
Calcium	CaO	400 sb, 290 m
Cerium	CeO_2	525 sh, 425 s vb
Chromium	CrO_2	(No bands 700–250)
	Cr_2O_3	625 s, 555 s, 435 w, 407 w
Cobalt	Co_3O_4	655 s, 635 sh, 562 s, 460 b sh, 350 w
Copper	CuO	610 m, 500 s, 410 m
	Cu_2O	615
Dysprosium	Dy_2O_3 (cubic)	550 s, 408 s vb, 320 m, 307 w, 284 w, 273 w
Erbium	Er_2O_3 (cubic)	563 s, 465 b sh, 367 s, 325 m, 285 m
Europium	Eu_2O_3 (monoclinic)	630 w, 510 sh, 365 s vb
Gadolinium	Gd_2O_3 (cubic)	535 s, 465 b sh, 350 s, 310 m, 297 w, 270 m
Gallium	βGa_2O_3	663 s, 450 sb, 364 m, 305 mb
Germanium	GeO_2 (hexagonal)	585 s, 550 s, 512 s, 332 s, 255 m
Gold	Au_2O_3	607
Hafnium	HfO_2 (monoclinic)	755 m, 645 m, 530 s, 450 sh, 425 s, 375 w, 350 sh
Holmium	Ho_2O_3 (cubic)	559 s, 470 b sh, 370 s, 325m, 310 sh 285 m
Indium	In_2O_3	(No bands 700–250)
Iron	αFe_2O_3	560 sb, 468 s, 370 sh, 325 s
	γFe_2O_3*	555, 468, 336
	Fe_3O_4	570 sb, 385 mb
Lanthanum	La_2O_3	644 w, 415 sb
Lead	PbO (orthorhombic)	500 m, 377 s, 300 s
	PbO_2 (tetragonal)	(No bands 700–250)
	Pb_3O_4	650 w, 525 s, 445 s, 380 s, 320 m
Lutetium	Lu_2O_3	570 s, 485 b sh, 382 s, 337 m, 297 m
Magnesium	MgO	445 vb
Manganese	MnO_2 (tetragonal)	615 s vb, 400 m, 335 w
	Mn_3O_4*	600, 475 393
Mercury	HgO (yellow)	588 s, 480 s
	HgO (red)	573 s, 475 s
Molybdenum	MoO_3*	660, 375, 300
Neodymium	Nd_2O_3*	655
Nickel	NiO	650 w, 465 s vb
Niobium	αNb_2O_5*	478, 295
	δNb_2O_5*	575, 455 sh, 357
Praseodymium	Pr_6O_{11}	655 m, 500 sh, 350 s vb
Ruthenium	RuO_2	(No bands 700–250)
Samarium	Sm_2O_3 (monoclinic)	640 w, 530 sh, 370 s vb
Scandium	Sc_2O_3	625 s, 525 sh, 425 s, 382 s, 382 m, 365 w, 343 w
Silicon	SiO_2 (dehydrated silica-gel)	460 b
	SiO_2 (α quartz)	775 m, 693 w, 510 sh, 450 s, 385 sh, 362 s, 257 m
Silver	Ag_2O	645 m, 540 s
Strontium	SrO_2	590 sb, 525 sh
Tantalum	αTa_2O_5	612 s vb, 450 b sh, 300 m
	βTa_2O_5*	575, 455 sh, 315
Thorium	ThO_2	645 sh, 310 s vb
Thulium	Tm_2O_3	565 s, 483 sh, 380 s, 335 w, 295 w
Tin	SnO* (tetragonal)	650, 480
	SnO_2	670 m, 610 sh, 312 s
Titanium	TiO_2 (anatase)	700 s vb, 525 s vb, 347 m
	TiO_2 (rutile)	695 sb, 608 sh, 423 w, 352 w
	Ti_2O_3*	650 (No bands 600–250)
Tungsten	WO_2	(No bands 700–250)
	WO_3*	355, 315
Vanadium	V_2O_5	595 s vb, 395 w, 288 w
Ytterbium	Yb_2O_3	569 s, 400 sh, 330 m, 322 sh, 296 m
Yttrium	Y_2O_3 (cubic)	561 s, 423 sh, 333 m, 325 sh, 300 m
Zinc	ZnO	450 vb
Zirconium	ZrO_2 (monoclinic)	745 m, 620 sh, 530 sh, 450 w, 420 w, 375 w, 360 sh
	ZrO_2 (cubic CaO stabilized)	490 vb

* Bands are poorly defined. s = high intensity, m = medium intensity, w = low intensity, b = broad, v = very, sh = shoulder (the intensity values should be compared only with bands in the same spectrum).

Sample preparation

It became apparent after recording the spectra of several of the metal oxides that a specific procedure would be necessary in order to obtain reproducible spectra from each sample. The ordinary mulling techniques were made more difficult by the varying hardness of the metal oxides. Difficulties of this nature have been encountered previously and various techniques have been studied [5, 19] in order to obtain representative and reproducible spectra. A review article by Duyckaerts [20] shows the effects that can occur in an infrared spectrum of solid substances. In this study 200 mg of each compound was ground dry in a mortar and pestle for approximately one minute with an additional one minute dry grind in a vial in a Wig-L-Bug vibrator. A plastic vial was normally used but requires caution with respect to contamination [21]. However, with 200 mg of oxide sample the concentration of the plastic in the sample remained small and did not prove to be a problem when recorded as a Nujol mull. Approximately 50 mg of the pre-ground sample was then mulled with Nujol. In some cases the thoroughly ground samples were also pressed into KBr disks for cross-checking purposes. In general the bands in the pellet spectra were less asymmetric than in the Nujol mull spectra. This was probably due to a decrease in scatter from the smaller amount of sample in the KBr matrix. The sample concentration ranged between 0·3 and 0·6 per cent for the compounds studied, which is small for this region of the infrared when compared to the 1 per cent normally used for organic materials.

Characteristics of the spectra

The instrument is capable of recording frequencies with an accuracy of $\pm 0{\cdot}5$ cm^{-1}. The band positions reported in this paper should be accurate to within $\pm 2{\cdot}0$ cm^{-1}. For very broad bands the error in determining the peak frequency would sometimes be greater than this value.

The study of inorganic compounds in the low frequency region of the infrared can be complex. Some theory about the spectra resulting from vibrational transitions in organic solids can be obtained from a comprehensive review by Mitra [22]. For the most part these data deal only with the practical applications of the low frequency spectra and no attempt is made to assign specific vibrational modes.

The effect of particle size on the infrared spectrum can be seen in Figs. 1 and 2. Figure 1 shows the spectrum of red mercuric oxide after various amounts of grinding. The initial curve represents the compound after it was ground a very short time with a mortar and pestle. The resulting spectrum has a very broad band with two possible maxima at 550 and 450 cm^{-1}. The sample was then ground in a vial using a Wig-L-Bug vibrator and curves A–C represent increased grinding periods. The final spectrum (curve C, 90 sec) shows two distinct peaks with maxima at 573 and 475 cm^{-1} indicating the two bands have shifted to a higher frequency by about 25 cm^{-1}. In contrast to this the yellow mercuric oxide gives two well defined peaks at 590 and 488 with only a very short grinding period. Using

[19] W. M. Tuddenham and R. J. Lyon, *Anal. Chem.* **32**, 1630 (1960).
[20] G. Duyckaerts, *Analyst* **84**, 201 (1959).
[21] N. T. McDevitt and W. L. Baun, *Appl. Spectrosc.* **14**, 135 (1960).
[22] S. S. Mitra, *Solid State Phys.* **13**, 1 (1962).

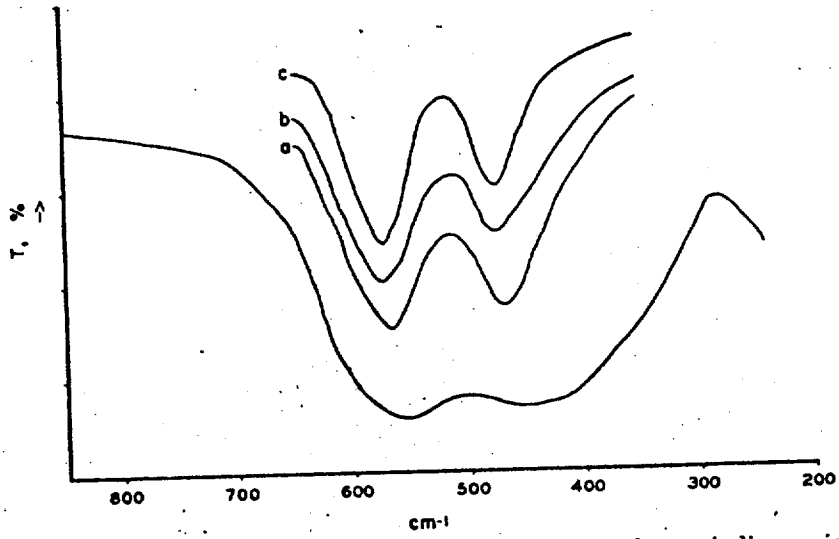

Fig. 1. Red mercuric oxide spectrum. Initial spectrum very short grinding period; a—20 seconds dry grinding with Wig-L-Bug vibrator; b—45 seconds; c—90 seconds.

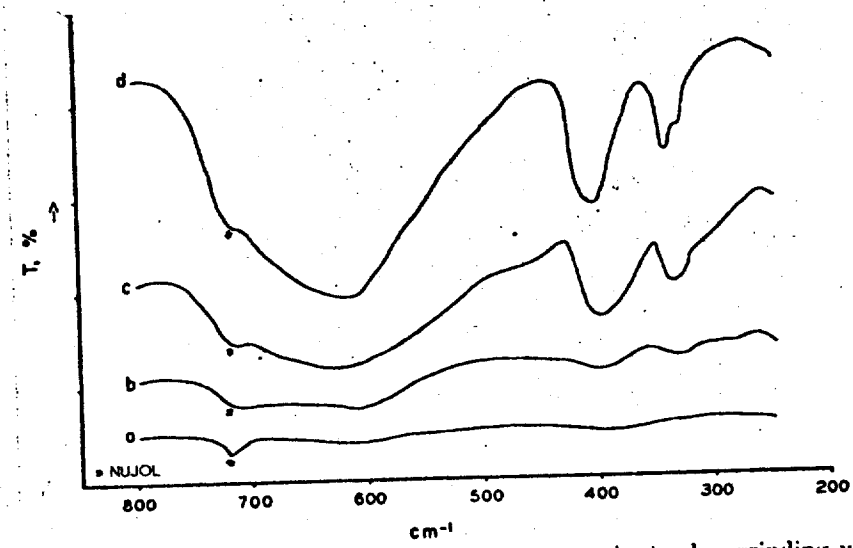

Fig. 2. MnO_2 spectrum. a—no grinding; b—one minute dry grinding with a mortar and pestle; c—one minute mortar and pestle plus one minute in a steel vial with a vibrator; d—one minute mortar and pestle plus two minutes in a steel vial with a vibrator.

the same procedure as described above for the red compound, the final spectrum of the yellow mercuric oxide had the same band shape as the initial curve and the peak frequencies are almost constant at 588 and 430 cm^{-1}. This is probably the most dramatic difference that will be seen among the metal oxides. All the data for these compounds show they have the same chemical composition, the same structure, and the most recent X-ray powder data by the National Bureau of Standards reports that the red oxide gives the same diffraction pattern as the yellow

compound. Photomicrographs of the two materials as received show the yellow mercuric oxide average particle size to be about 5 μ, while the red mercuric oxide average particle size is nearly 20 μ. Therefore, the particle size of the yellow compound is approximately $\frac{1}{5}$ the wavelength of its absorption bands while the particle size of the red oxide is just about equal to its maximum absorption wavelength. It appears that this is the reason for the difference in their infrared spectra. The increase in frequency of the red oxide absorption bands on grinding could be attributed to particle size or possibly to a lattice strain set up in the molecule through

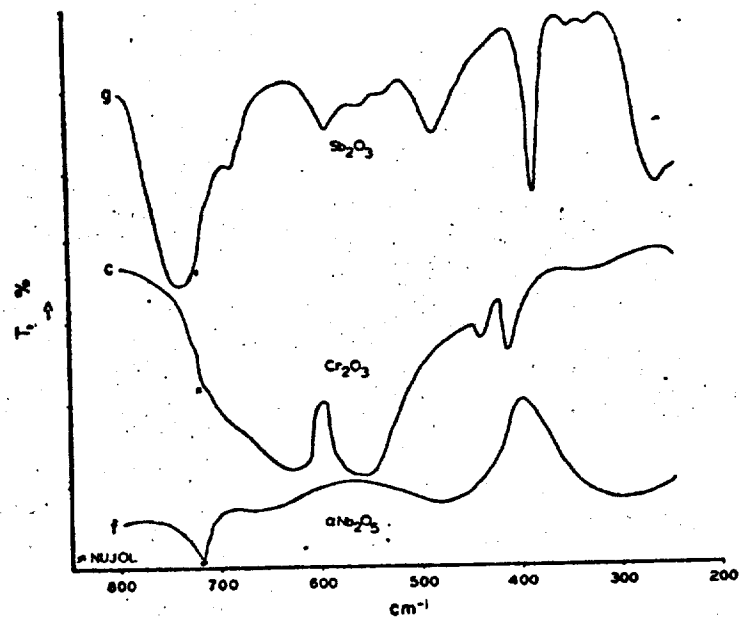

Fig. 3. Examples of spectra that can be obtained from metal oxides. g—Sb_2O_3; e—Cr_2O_3; f—αNb_2O_5.

grinding. The spectra for both compounds show two symmetrical absorption bands with the higher frequency band the more intense in each case.

Unlike the red mercuric oxide, the initial spectrum of MnO_2 (Fig. 2) gave very little indication that any characteristic absorption bands were present. This compound is shown as the extreme example of the necessary amount of grinding required to obtain a spectrum. More than 120 sec of grinding in a steel vial was required to thoroughly diminish the particle size of the sample so that a representative curve could be obtained. In most cases grinding for this length of time usually causes some undesired changes and possible phase transformations [23]. If a representative curve cannot be obtained after 120 sec of grinding the compound probably does not have a characteristic absorption spectrum in this region.

The general appearance of spectra obtained from metal oxides can be classified in three categories, i.e., good, average and poorly defined. The curves shown in Fig. 3 represent examples of these types. Curve G, Sb_2O_3, illustrates a good spectrum (about 10 per cent of the spectra studied), curve E, Cr_2O_3, is an average

[23] F. Dachille and R. Roy, *Nature* 186, 34 (1960).

spectrum, while F, αNb_2O_5, represents a poor spectrum (about 10 per cent of the compounds studied).

Rare-earth oxides

The spectra for a group of rare-earth oxides [24] (Lu_2O_3, Yb_2O_3, Tm_2O_3, Er_2O_3, Y_2O_3, Ho_2O_3, Dy_2O_3, Gd_2O_3) give a well defined characteristic band in the region 570–530 cm^{-1}. Figure 4 shows a spectrum of a typical type C rare-earth, ytterbia, and a plot of frequency against unit cell dimension for the type C (cubic) oxides.

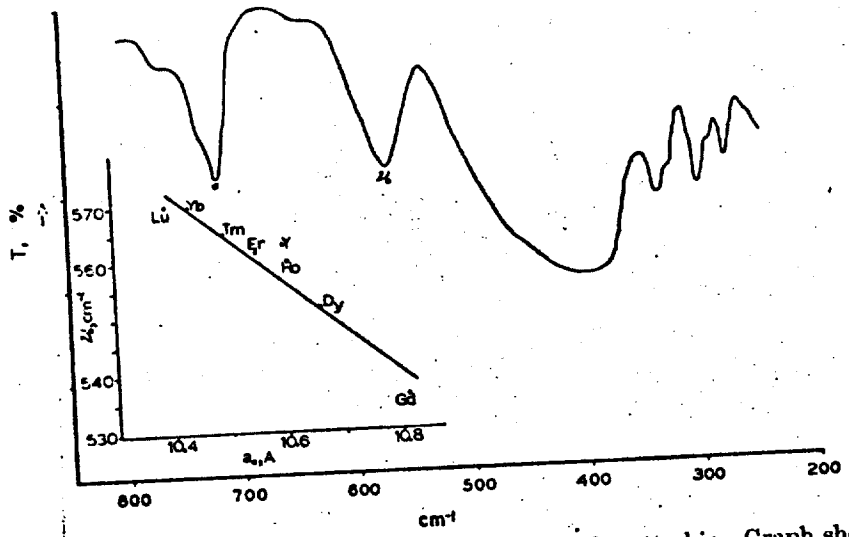

Fig. 4. Spectrum of a typical type C rare-earth oxide, ytterbia. Graph shows a plot of frequency against unit cell dimension of type C oxides.

The linear relation indicates a correlation with the high frequency band and the unit cell size. The type A (hexagonal) and type B (monoclinic) rare-earth oxides give different spectra from one another and the type C compounds. The optical properties [25] of the rare-earth oxides are becoming of interest, and distinguishing between one crystal structure and another can be of importance. An example of this is gadolinium sesquioxide, which can exist as type A, B, or C. In this study the infrared spectrum indicated the compound was of type C, removing any uncertainty as to its crystal structure.

Polymorphism

One of the more important determinations of this study is the role that polymorphism plays in characterizing the infrared spectrum of a compound. An oxide spectrum can be misleading if only the molecular formula is given when the particular compound can exist in several forms. An example of such an ambiguity

[24] W. L. Baun and N. T. McDevitt, *J. Am. Ceram. Soc.* **46**, 294 (1963).
[25] S. S. Batsanov, G. N. Grigor'eva and N. P. Sokolova, *J. of Structural Chem.* (*U.S.S.R.*) **3**, 323 (1962).

can be seen in spectrum 159G (TiO_2) in Reference [3]. Titanium dioxide has two commonly occurring crystal structures, anatase and rutile, and each gives a different infrared spectrum. These spectra can be seen in Fig. 5. The anatase structure has a very characteristic band at 347 cm^{-1}, whereas the rutile spectrum is quite different. Spectrum 159G in Reference [3] can now be identified as TiO_2 with an anatase structure. The infrared spectra of the polymorphic forms of TiO_2 have been studied in the rock salt region [6]. However, the results left much to be desired as a quick means of identifying the above compounds.

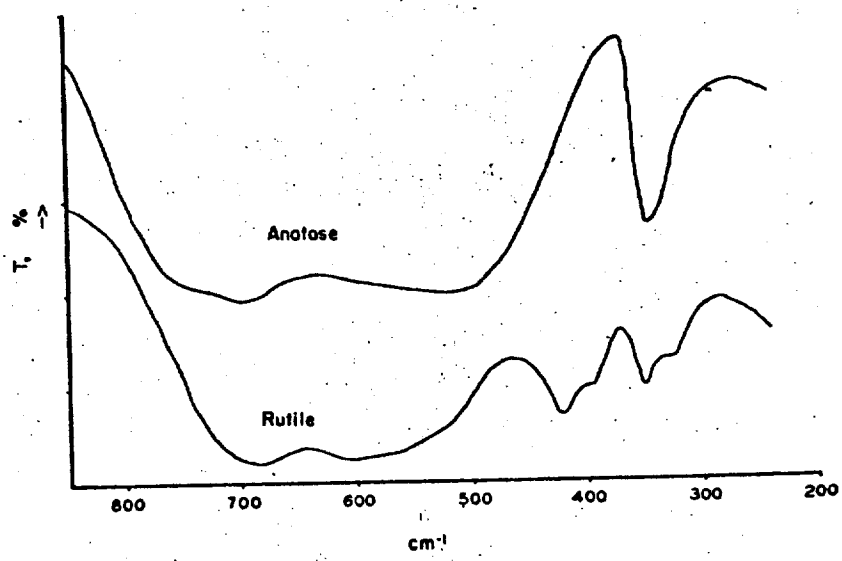

Fig. 5. Spectra of the polymorphic forms of titanium dioxide.

The present study includes several other polymorphic compounds namely, αFe_2O_3 and γFe_2O_3, αNb_2O_5 and δNb_2O_5, and αTa_2O_5 and βTa_2O_5. Their frequencies can be obtained from Table 1, and although the fundamental structural differences are in most cases slight, the infrared spectra serve to distinguish the various polymorphic forms.

Comments on individual compounds

An interesting pair of compounds are zirconia and hafnia. The metals of these compounds have very similar chemical properties in spite of the large difference in their atomic weights. It is understandable then that ZrO_2 and HfO_2 have similar properties such as a monoclinic crystal structure at room temperature. This similarity can be seen in the infrared spectrum where each curve shows seven absorption bands with closely related frequencies. However, both compounds can be easily identified from the different band shapes as can be seen in Fig. 6 (zirconia and hafnia show no absorption bands in the rock salt region of the infrared). Since the concentration in KBr was less than 1 per cent it is evident that these compounds absorb strongly in this region. Application of this region to research on ZrO_2 has

been discussed elsewhere [26]. A typical example can be seen by comparing the frequencies of monoclinic ZrO_2 to the cubic form (stabilized with CaO) in Table 1.

Eight of the spectra listed in Table 1 are shown as having "No bands" in the region studied. Several of these compounds (BaO_2 and In_2O_3), however, do show some indication of a beginning of a large broad band with the high frequency side starting around 450 cm^{-1}. The BeO spectrum shows only the low frequency side of a very broad band that has its maximum absorption frequency at approximately 830 cm^{-1}. PbO_2 and CdO are unusual compounds in that they have no apparent

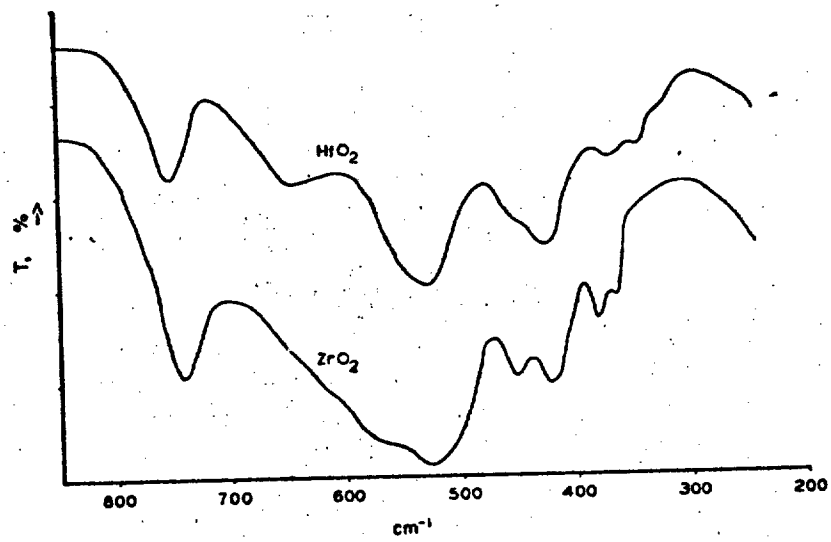

Fig. 6. Spectra of two similar compounds, hafnia and zirconia.

absorption bands between 4000 and 240 cm^{-1}. The infrared transmission of each material varies with frequency, but in both cases the change occurs over a very wide range. The concentration of the samples was greater than 1 per cent in KBr so the absence of absorption bands cannot be considered as a lack of sample in the matrix.

Comparison of individual studies in the literature with the data presented here is in good agreement with a few exceptions. PARODI [16] reports CuO as having one band at 263 cm^{-1} while the CuO spectrum in this paper shows bands at 610, 500 and 410 cm^{-1}. Analysis of our compound showed it to be at least 95 per cent monoclinic CuO. Other C.P. grades labeled CuO were checked by X-ray diffraction and found to contain large amounts of Cu_2O. Without knowing the purity of Parodi's CuO the above discrepancy is not resolvable at this time. Much of the data on oxides reported by VRATNY and co-workers [27] for the region 5000–416 cm^{-1} do not agree with frequencies reported here. For example, the HgO spectrum reported in Reference [27] shows only one strong band at 574 cm^{-1}. This compound

[26] N. T. McDEVITT and W. L. BAUN, *J. Am. Ceram. Soc.* (In press).
[27] F. VRATNY, M. DILLISO, F. GUGLIOTTA and C. N. R. RAO, *J. Sci. Ind. Research* **20B**, 590 (1961).

is probably the red HgO and proper sample preparation was not taken into consideration (see Fig. 1). In this same paper the spectrum of Sb_2O_3 is poorly presented, because the spectrum from the same compound shows well defined bands in Ref. [3] and also this paper (Fig. 3).

Conclusion

In most cases the transmission spectra of the metal oxides exhibited broad absorption bands in the region studied. Therefore, sample preparation with respect to particle size is the most important factor when recording the infrared spectra of these compounds. The larger the particle size of the material the wider the apparent band width becomes. The data from this study indicates the particle size should be 10 μ or smaller for this region of the infrared in order to obtain a representative spectrum. Small differences in crystal structure can also be detected, giving rise to a valuable tool for the study of polymorphism and an aid in the research of refractory metal oxides.

The data presented here should serve as a powerful qualitative tool for the identification of and research on metal oxides.

Acknowledgements—The authors wish to thank F. F. Bentley for his interest and discussions on the spectra used in this study.

Dr. Moats prepares sample for heating (0870C762-10).

destroying SALMONELLA by heat

The material carrying this bacteria can affect its susceptibility to heat

A VARIETY of chemical constituents in food protect *Salmonella anatum*, a food poisoning bacteria, from destruction by heat—a finding that may help researchers find ways to reduce the incidence of sickness caused by this organism.

In studies at Beltsville, Md., ARS chemist William A. Moats, microbiologist Roger Dabbah, and technician Virginia Mae Edwards added *Salmonellae* bacteria to various test materials, which were heated to 131° F., for 35 minutes. Bacterial readings were then made.

Some materials rendered bacteria more susceptible to destruction by heat. Glucose and sodium citrate, for example, reduced *Salmonellae* survival to only nine bacteria or less per milliliter of test material.

Other materials protected *Salmonellae*. These included a commercial ice cream stabilizer, various carbohydrates, proteins, and some amino acids. The carbohydrates sucrose, rhamnose, and mannitol, for instance, had *Salmonellae* survival rates ranging from 31,000 to 58,000 per ml. But milk was the most dramatic example. "Milk protected bacteria from heat better than anything we tested," Dr. Moats reported.

Milk commercially sterilized by heating for a few seconds at very high temperatures was selected for testing because it more closely resembles fresh milk from the cow than does milk sterilized by autoclaving for 15 minutes. Autoclaving, a method of heating by steam under pressure, was used in many of the previous tests.

The heat treatment employed in the ARS experiments was considerably less stringent than treatments used for commercial pasteurization of milk; therefore, the tests do not imply that *Salmonellae* can survive pasteurization.

In subsequent tests, the sterilized milk was subjected to autoclaving, which "largely destroyed the protective effect previously existing in the sterilized milk," Dr. Moats said. "Bacterial survival was 1,000-fold less than in unautoclaved milk. Unfortunately, much of the past data on heat resistance of bacteria in milk has been obtained using autoclaved milk and therefore needs to be reevaluated."

Of the 17 amino acids that were also tested, alanine gave the most protection against heat. About 35,000 bacteria per ml. of alanine test material survived exposure to the experimental heat treatment. Ten other amino acids also gave considerable protection in the tests.

"Although there may be other explanations for these results," Dr. Moats said, "the most attractive theory is that these food constituents react with heat-sensitive proteins in the bacterial cell, stabilizing the proteins to heat." ■

Methyl ester of γ-phenyltetrahydrofuranon-2-γ-carboxylic acid VI from acid VIIIb

Two grams of 3-hydroxy-3-allophanyl-3-phenylpropane-1-carboxylic acid (VIIIb) were dissolved in 20 ml of 4% methanolic solution of hydrogen chloride and set aside at room temperature for 4—5 days. The methanol was then evaporated under reduced pressure, and the residue was extracted with ethyl ether and filtered to separate ammonium chloride. After some time, a product with mp 54—56° crystallized from the ether filtrate, which was identical with the methyl ester of γ-phenyltetrahydrofuranon-2-γ-carboxylic acid (VI).

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THE USE OF ION EXCHANGE FOR THE QUANTITATIVE ASSAY OF MIXTURES OF CITRATE AND CITRIC ACID

by

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A method for the quantitative assay of mixtures of NaOH and citric acid with pH 4·95 has been developed. The quantitative composition and concentration of the solution correspond to a mixture of trisodium citrate with the formula $C_6H_5Na_3O_7 \cdot 2H_2O$ and citric acid with the formula $C_6H_8O_7 \cdot H_2O$ contained in the blood preserving fluid "ACD". The determinations were made on the cation exchanger Zerolit-225 and the anion exchanger Permutit-ESB. An important parameter of the method of sorption on cation exchange resin consists in washing it free from adsorbed citric acid with 96% ethyl alcohol. Eluates from the cation exchanger column were filtrated with NaOH solution, in the presence of phenolphthalein, the titer of which was adjusted to citric acid. Total content of citric acid in the sample was calcuated from the titration results. Effluents from the anion exchanger column were titrated with hydrochloric acid in the presence of methyl orange. Citrate can be calculated from the amount of NaOH found in the sample. The amount of free citric acid is the difference between the total amount of acid in the effluent from the cation exchanger column and amount of acid bound by the previously determined citrate.

The subject of this study was dictated by the need of checking blood preserving fluids. Besides preserving fluids, blood banks produce a wide variety of injection fluids. Only the latter are checked by qualitative and quantitative chemical methods for their purity and composition. For preserving fluids, such methods are not available, and they are examined only biologically and bacteriologically. As a first step in the development of a method for chemical control of these fluids, the problem of quantitative assay of mixtures of citric acid FP III and sodium citrate FP III has been undertaken. The proportions were those used in the "ACD" preserving fluid. An attempt has been made to solve this problem by the use of ion exchangers. Positive results of the experiments were expected to solve the problem of the quantitative analysis of these fluids, and of their differentiation from other electrolyte solutions.

MATERIAL AND METHODS

The object of this study was a mixture of solutions of NaOH and citric acid with known concentrations. The composition of the mixture was similar to that in the ACD preserving fluid:

trisodium citrate FP III	13·2 g
citric acid FP III	4·8 g
glucose FP III	14·7 g
aqua pro injectione ad	1000 ml.

Glucose was not taken into account when preparing the mixture, because as a non-electrolyte it does not influence the experimental results.

The solution of the problem by means of ion exchangers was based on the determination of sodium hydroxide in the post-anion exchanger effluent and calculation from the result of the amount of citrate in the examined volume of solution.

It was expected that by titration with sodium hydroxide the whole amount of bound and free citric acid could be determined. Substracting the amount of citric acid corresponding to the amount of citrate previously determined, the whole amount of acid contained in a known volume of solution in the free state can be calculated.

The following cation exchangers were used for sorption:

8×210 mm; Zerolit-225 [H^+]. Flow rate 4 ml cm^{-2} min^{-1}
8×125 mm; Kastel-C-100 [H^+]. Flow rate 3 ml cm^{-2} min^{-1}

and the following anion exchangers:

8×180 mm; Permutit-ESB [OH^-]. Flow rate 2 ml cm^{-2} min^{-1}
8×180 mm; Permutit-E [OH^-]. Flow rate 3 ml cm^{-2} min^{-1}.

Descending sorption in glass columns was performed. The height of the beds was measured after regeneration and washing with water.

Assuming that 10-ml samples of ACD were used for analysis, the theoretical content of citric acid in this volume was calculated:

0·312 citrate FPIII	corresponds to	0·09432 g citric acid FP III
		+0·04800 g free citric acid
Total		0·14232 g citric acid FP III

The content of sodium in 10 ml of ACD is corresponding to 13·464 ml 0·1 N NaOH.

The sorption mixture was prepared from:

10 ml of citric acid solution containing 0·1423 of pharmacopoeial substance, which on titration in the presence of phenolphthalein utilized 19·2 ml of NaOH solution with titer Z_1, and 13·5 ml of 0·1 N NaOH, which on titration in the presence of methyl orange utilized 16·1 ml of HCl solution with titer = Z_2.

After regeneration of the cation exchangers with 5% HCl and washing with water, 23·5 ml of the above mixture was determined by the following methods:

A. After sorption, washing with five 10-ml portions of water and determination of citric acid in the effluent by means of sodium hydroxide solution in the presence of phenolphthalein.

B. Before sorption, 100 ml of 1 N citric acid was passed through the columns; after washing the ion exchangers with water to neutral reaction of the effluent, sorption was carried out. After sorption the exchangers were washed with five 10-ml portions of water and the effluent was titrated as above.

C. After sorption the columns were washed three times with 10 ml of water, then three times with 10 ml of 96% alcohol, and the effluent was titrated as above.

Preparation for sorption and sorption on anion exchangers were carried out as follows: after regeneration with 3% NaOH, washing with water to neutral reaction of the effluent; sorption of 23·5 ml of the mixture of NaOH and citric acid, followed by six washings of the deposits with 10-ml portions of water. NaOH was titrated in the effluent by means of hydrochloric acid in the presence of methyl orange.

RESULTS AND DISCUSSION

Employing method A, the total amount of citric acid applied to the top of the column was not found in the post-cation exchange effluents:

Zerolit-225	92·0%
,,	92·4%
,,	92·8%
Kastel-C-100	90·6%
,,	90·3%
,,	90·4%

The high percentage of error was probably due to retention of the large citrate ions on the resins as a result of adhesion. An attempt was made to eliminate this phenomenon by saturating the exchangers with citric acid before sorption, according to method B. However, the results were weighted by a large error, as in method A.

By using method C, it was hoped to wash out the citric acid from the cation exchange beds by disturbing the equilibrium of the adhesive system exchangers—citric acid. The large excess of alcohol was expected to occupy the adhesive centers of the resin, freeing the acid. The expectations were confirmed when Zerolit-225 was used. The volumes of NaOH solution used to titrate the effluent from the cation exchangers are given in Table 1.

As can be seen, in method C the strongly acid cation exchanger Zerolit-225 fulfills the conditions and can be used for sorption of Na ions since it allows citric acid to pass into the effluent quantitatively. When the ion exchange resin was not washed with alcohols, results were too low.

Kastel-C-100 cannot be used for this purpose. This cation exchanger is too weakly acid and does not sorb sodium ions quantitatively at the pH of the preserving fluid.

Permutit-ESB, a strongly alkaline anion exchanger, sorbs 100% of citrate ions, giving an equivalent amount of NaOH in the effluent, which can be determined by titration with hydrochloric acid in the presence of methyl orange. The results show that this ion exchanger resin, without regeneration of the beds, gives identical titration results in 4 successive sorptions, indicating its high break-through capacity. The result can be converted by calculation to

Table 1. Results of titration of acid effluents

Exchanger	NaOH solution utilized for titration of the effluent (ml)	Per cent error
Zerolit-225	19·2	0
"	19·2	0
"	19·2	0
"	19·2	0
"	19·2	0
"	19·2	0
Kastel-C-100	16·0	—16·7
"	16·0	—16·7
"	16·0	—16·7

Table 2. Results of sorption on anion exchangers

Exchanger	HCl solution utilized for titration of the effluent (ml)	Per cent error
Permutit-ESB	16·1	0
"	16·1	0
"	16·1	0
"	16·1	0
Permutit-E	14·9	—7·5
"	14·0	—13·0
"	9·8	—39·0

content of sodium citrate FP III. This ion exchanger fulfills the purpose for which it was used (Table 2).

Permutit-E, a weakly alkaline anion exchanger, does not sorb citrate ions quantitatively at this pH, as shown by the results of quantitative determination of NaOH in the effluent. Hence, this anion exchanger cannot be used under the experimental conditions in this study.

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Although there was no change in the total incidence of hospital-acquired staphylococcal infection, the number of infections caused by the Type 54 organism increased whereas the number of those caused by Type 80/81 decreased.

Increased use of neomycin for preoperative preparation of the bowel was associated with and may have been responsible for emergence of the Type 54 strain.

The possible implications of the change in type of staphylococcus causing infection in the hospital is discussed.

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HEMODYNAMIC EFFECTS OF INTRAVENOUSLY ADMINISTERED SODIUM CITRATE*

John P. Bunker, M.D.,† Henrik H. Bendixen, M.D.,‡ and Anna J. Murphy, B.S.

BOSTON

Marked elevations of serum citrate have been reported to occur in man during multiple rapid transfusions of citrated blood.[1-3] The possibility of harmful effects is well recognized but often discounted.[2,4] In an attempt to separate the circulatory effects of citrate from many other potentially adverse effects of massive transfusion, we have studied the hemodynamic effects of intravenous infusions of sodium citrate in normal, lightly anesthetized man and in the dog.

Methods

Clinical Material

Six patients, 5 females and 1 male, were studied. Their ages were thirty-one to forty-four, with an average of thirty-eight years. All were undergoing ligation and stripping of varicosities of the lower extremities, and all were free of systemic disease according to the history and physical examination. Pentobarbital, 100 mg., and atropine, 0.6 mg., were administered intramuscularly approximately forty-five

*From the Anesthesia Laboratory of the Harvard Medical School at the Massachusetts General Hospital.

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tracheally administered nit
D-tubocurarine was admin
doses adequate to achieve a
tilation was maintained w
pressure by means of th
Meperidine, 45 to 100 mg
divided doses, was used to s
in 3 of the 5 patients. The s
1) received thiopental for

Table 1. *Clinical an*

Patient	Weight	Amount of Citrate Infused	Duration of Infusion	Ra of fus
	kg.	gm.	min.	mg. kg. mi
L.V.	60	4.0	16	4.2
M.H.	72	(6.0)	17	(5.0
J.D.	71	5.6	17	5.3
M.S.	73	5.8	9	5.8
H.E.	49	6.9	19	7.4

*Observations before infusion, at tim
†After receiving calcium chloride intr

choline to facilitate endotrac
by light nitrous oxide, ox
anesthesia with spontaneous
tients a Cournand needle
artery of both arms to allo
measurement of cardiac o
analyses. A No. 17-gauge p
tracath) was introduced int
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Dye for cardiac-output det
through this catheter. A se
duced into the superior ve
jugular vein to measure cen

Laboratory Animals

Nine dogs were studied. L
tained with intermittent do
cent, given intravenously. A
tube was introduced, and
oxygen and air was adm
positive pressure with a Ha
pirator Pump No. 607.† La
(internal diameter of 1.68

*Manufactured by Air-Shields, Inco
†Harvard Apparatus Company, Inc

minutes before induction of anesthesia. In 5 patients anesthesia was induced with intravenously infused thiopental, 2.5 per cent, and maintained with endotracheally administered nitrous oxide and oxygen. D-tubocurarine was administered intravenously in doses adequate to achieve and maintain apnea. Ventilation was maintained with intermittent positive pressure by means of the Jefferson Ventilator.* Meperidine, 45 to 100 mg., given intravenously in divided doses, was used to supplement the anesthesia in 3 of the 5 patients. The sixth patient (L.V., Table 1) received thiopental for induction and succinylcholine to facilitate endotracheal intubation, followed by light nitrous oxide, oxygen and diethyl ether anesthesia with spontaneous respirations. In all patients a Cournand needle was placed in the radial artery of both arms to allow sampling of blood for measurement of cardiac output and for chemical analyses. A No. 17-gauge polyethylene catheter (Intracath) was introduced into the basilic vein at the antecubital fossa and advanced approximately 40 cm. Dye for cardiac-output determinations was injected through this catheter. A second catheter was introduced into the superior vena cava via the external jugular vein to measure central venous pressure.

TABLE 1. *Clinical and Hemodynamic Effects of Intravenously Administered Sodium Citrate in Man.**

Patient	Weight	Amount of Citrate Infused	Duration of Infusion	Rate of Infusion	Serum Calcium	Serum Citrate	Serum Magnesium	Serum Total Protein	Calculated Ionized Calcium	pH	Mean Arterial Blood Pressure	Pulse Pressure	Cardiac Output	Pulse Rate	Stroke Volume	Work of Left Ventricle	QTc
	kg.	*gm.*	*min.*	*mg./kg./min.*	*milli-mols/liter*	*milli-mols/liter*	*milli-mols/liter*	*gm./100 ml.*	*milli-mols/liter*		*mm. Hg.*	*mm. Hg.*	*liters/min.*		*ml.*	*kg. meters/min.*	
L.V.	60	4.0	16	4.2	2.25	0.12	0.75	—	1.04	—	65	45	4.8	80	60	4.2	0.427
					2.1	2.4	0.5	6.1	0.50	—	40	30	5.1	110	46	2.7	0.517
					2.05	0.72	0.7	5.9	0.85	—	45	40	5.0	80	63	3.0	0.443
M.H.	72	(6.0)	17	(5.0)	2.28	0.14	0.9	6.3	1.06	—	115	40	4.2	89	47	6.5	0.450
					—	3.5	—	—	(0.47)	—	83	20	3.7	102	36	4.1	0.592
					3.40†	3.3	0.6	5.5	0.85	—	105	45	6.8	105	65	9.6	0.523
J.D.	71	5.6	17	5.3	—	—	—	—	—	—	93	40	4.9	80	61	6.1	0.446
					2.13	3.5	0.9	5.8	0.41	7.56	70	20	3.4	92	37	3.2	0.524
					2.15	0.78	0.95	6.1	0.85	7.59	90	30	4.3	75	57	5.2	0.447
M.S.	73	3.8	9	5.8	2.25	0.16	0.9	6.3	1.04	7.58	92	35	3.0	74	41	3.7	0.462
					2.08	3.6	0.7	6.0	0.37	7.47	82	20	2.1	98	21	2.3	0.660
					2.75†	1.9	0.65	5.5	0.88	7.55	93	40	4.7	78	60	5.9	0.491
H.E.	49	6.9	19	7.4	2.28	0.10	1.1	6.0	1.11	7.63	95	35	2.8	60	47	3.6	0.485
					2.10	4.1	0.75	5.7	0.35	7.59	66	24	3.5	95	37	3.1	0.643
					2.13	1.5	0.95	5.8	0.70	7.63	77	34	3.7	72	51	3.8	0.552

*Observations before infusion, at time of peak elevations in serum citrate & during recovery.
†After receiving calcium chloride intravenously.

Laboratory Animals

Nine dogs were studied. Light anesthesia was maintained with intermittent doses of thiopental, 2.5 per cent, given intravenously. A large, cuffed endotracheal tube was introduced, and through it a mixture of oxygen and air was administered by intermittent positive pressure with a Harvard variable-speed respirator Pump No. 607.† Large polyethylene catheters (internal diameter of 1.68 mm.) were introduced and tied into femoral arteries and veins bilaterally and into the left carotid artery and external jugular vein. A thoracotomy was performed in the left fifth or sixth costal interspace. The pericardium was opened widely; a Walton–Brodie strain gauge‡ was sutured to the left ventricle approximately 2 to 4 cm. from the apex. Care was taken to observe the precautions for use and interpretation of this instrument outlined by Boniface et al.[5] and by Cotten and his associates.[6,7] Large polyethylene catheters were introduced and tied into the apex of the left ventricle and, in 3 animals, a major pulmonary artery. The pericardium was loosely closed, and the chest closed tightly in three layers. A chest catheter was left in the incision in several of the animals to allow removal of air by syringe. In 2 animals total sympathetic block was produced with repeated epidural injections of procaine, 0.4 per cent, or lidocaine (Xylocaine), 0.5 or 1.0 per cent, with the use of the technic described by Brewster, Isaacs and Andersen.[8]

Hemodynamic Measurements

Cardiac output was determined by the dye-dilution method of Hamilton et al.[9] For 3 patients the dilution curve was determined from samples collected at one-second intervals in a rotating disk-fraction collector. A Coleman, Jr., Spectrophotometer with microcuvettes was used to measure dye concentration. In the remaining 3 patients and in all the dogs dye-dilution curves were obtained with the Colson densitometer and recorded with a Sanborn Model 150 four-channel direct-writing recorder, the blood being returned to patient or dog. Cardiac output, work of the left ventricle, total peripheral resistance and mean transit time were calculated by means of

*Manufactured by Air-Shields, Incorporated, Hatboro, Pennsylvania.
†Harvard Apparatus Company, Incorporated, Dover, Massachusetts.

‡Made by Mr. Oliver T. Brodie, Charleston, South Carolina.

standard equations.[10] Electrocardiogram, arterial pressure and central venous pressure were recorded in man and dog. Changes in QT interval were corrected for changes in pulse rate:

$$QT_c = \frac{QT\ (\text{sec.})}{\sqrt{RR\ (\text{sec.})}}.$$ [11,12]

Full-range and, in most experiments, condenser-integrated mean pressures were obtained with Sanborn variable capacitance transducers (Model 267B) and recorded. In the animal experiments a second four-channel recorder was used to allow recording of the contractile force of the left ventricle and pressures in the left atrium, pulmonary artery and left ventricle.

Chemical Determinations

Serum citrate was measured by Elliott's[13] modification of the method of Natelson, Pincus and Lugovoy. Serum calcium levels were determined by the method of Fiske and Logan[14] and serum magnesium by the method of Garner.[15] Total protein was calculated from the optical density at 280 millimicrons in a Beckman model DU spectrophotometer. Serum pH was determined with the use of a Beckman glass electrode and Beckman model G pH meter. Ionized calcium was calculated from total calcium, total protein, citrate and magnesium, the equations of McLean and Hastings[16] being used.

Experimental Procedure

Anesthesia was maintained at as light a level as possible. No additional thiopental, meperidine or curare was administered to patients, or thiopental to dogs, after the beginning of the infusion of sodium citrate. When blood pressure, depth of anesthesia and ventilation were stable and satisfactory, sodium citrate was infused as 2.5, 10 or 20 per cent solution at maximum rates between 3.7 and 7.4 mg. of citrate per kilogram of body weight per minute in patients. A fall in blood pressure in a patient was treated by cessation of the infusion or by intravenous administration of calcium chloride. In the dog treatment consisted of intravenous infusion of calcium chloride, epinephrine or acetylstrophanthidin. Maximum rates of infusion of citrate in the dog varied between 5 and 17 mg. per kilogram per minute.

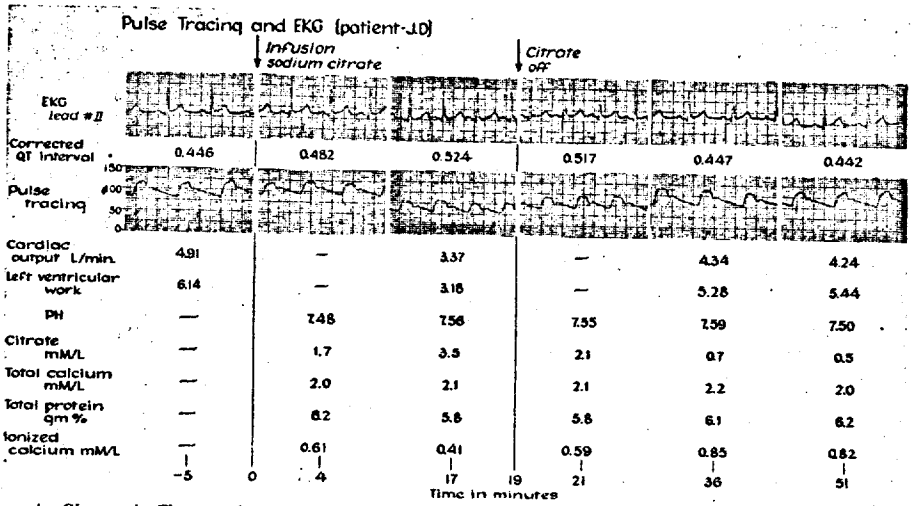

FIGURE 1. *Changes in Electrocardiogram, Radial Arterial Blood Pressure, Cardiac Output and Work of the Left Ventricle in a Twenty-nine-Year-Old Man during a Nineteen-Minute Infusion of 2.5 per Cent Sodium Citrate at a Rate of 5.3 Mg. of Citrate per Kilogram of Body Weight per Minute.*

Anesthesia consisted of intravenous administration of thiopental, meperidine and D-tubocurarine and endotracheal administration of nitrous oxide and oxygen, with controlled respiration (Jefferson ventilator). Surgery comprised bilateral ligation and stripping of varicosities of the long saphenous veins. The paper speed was 2.5 cm. per second. Note the prolongation of the QT interval, increase in pulse rate and fall in arterial-blood and pulse pressures during the citrate infusion and the gradual recovery after the infusion was stopped.

RESULTS

Patients (Table 1 and Fig. 1)

Maximum concentrations of serum citrate varied between 2.4 and 4.1 millimols per liter (46 and 77 mg. per 100 ml.). Serum total calcium remained essentially unchanged, whereas calculated ionized calcium fell from normal levels of 1 millimol to 0.5 millimol per liter or less in 5 of 6 patients. Mean arterial-blood pressure fell 16 to 38 per cent in these

5 patients, pulse pressure fell 37 to 50 per cent, and there was accompanying electrocardiographic evidence of hypocalcemia (prolongation of corrected QT interval). Cardiac output fell 12, 28 and 31 per cent in 3 patients, remained essentially unchanged in 1 and rose in 1. Pulse rate rose in all 5 patients, markedly in 3. Calculated stroke volume fell in all 5 and closely mirrored changes in pulse pressure. Work of the left ventricle declined markedly in 4 of the 5. Central venous pressure rose 2.5 and 3 mm. of mercury in 2 patients (L.V. and H.E.) but was unchanged in the remainder. Alterations in blood pressure, cardiac output, work of the left ventricle and electrocardiogram were corrected promptly by stopping the infusion of citrate or by the administration of calcium chloride. There were no hemodynamic changes in the sixth patient (not included in Table 1), who received the slowest infusion (3.7 mg. per kilogram of body weight per minute) and in whom maximum citrate levels remained at approximately 1.6 millimols per liter (30 mg. per 100 ml.).

Animals (Table 2 and Fig. 2)

Elevations in serum citrate levels of the same order of magnitude as those observed in the patient experiments (2.7 to 4.2 millimols per liter) were accompanied by hypotension, which was variable and usually mild, a mild to moderate fall in cardiac output, electrocardiographic evidence of hypocalcemia (depressed T wave and pulsus alternans), depression of the contractile force of the left ventricle of 20 to 90 per cent, as measured by the Walton-Brodie strain gauge, a rise in the end-diastolic pressure of the left ventricle of 3 to 10 mm. of mercury, a rise in central venous (external jugular) pressure of 1 to 9 mm. of mercury, a marked prolongation of mean transit time and, in most animals, generalized fine muscle tremors. Calculated total peripheral resistance rose in 2 dogs, was unchanged in 2 and fell in 1. Mean pulmonary-artery pressure, measured in 3 animals, rose 4 to 9 mm. of mercury in 2 (Fig. 2) and remained unchanged in the third. Further increases in citrate levels led to progressive circulatory deterioration; maximum serum concentrations tolerated without treatment varied between 2.7 millimols per liter (50 mg. per 100 ml.), in dog No. 12, and 10.0 millimols per liter (190 mg. per 100 ml.), in dog No. 2. Calculated ionized calcium at the time of circulatory collapse or death varied from 0.55 to 0.16 millimol per liter. In a single dog, not included in Table 2, calcium chloride infused before citrate provided the expected protection against these hemodynamic changes.

There was a marked increased sensitivity to infused citrate in 2 dogs (not included in Table 2) in which total sympathetic block was produced with epidural administration of procaine or lidocaine. Dog No. 8 succumbed at a serum level of 3.2 millimols per liter (62 mg. per 100 ml.). At a serum citrate level of 1.5 millimols per liter (28 mg. per 100 ml.) the mean blood pressure of dog No. 9 fell to 20 mm. of mercury but returned to 115 mm. of mercury at once when an infusion of 0.5 microgm. of epinephrine per kilogram of body weight per minute was begun,

TABLE 2. *Chemical and Hemodynamic Effects of Intravenously Administered Sodium Citrate in the Dog.**

Dog No.	Sex	Weight	Amount of Citrate Infused	Duration of Infusion	Rate of Infusion	Serum Calcium	Serum Citrate	Serum Magnesium	Serum Total Protein	Calculated Ionized Calcium	Mean Arterial Pressure	End-Diastolic Pressure in Left Ventricle	Mean Central Venous Pressure	Contractile Force of Left Ventricle	Cardiac Output	Work of Left Ventricle	Mean Transit Time
		kg.	*gm.*	*min.*	*mg./kg./min.*	*millimols/liter*	*millimols/liter*	*millimols/liter*	*gm./100 ml.*	*millimols/liter*	*mm. Hg.*	*mm. Hg.*	*mm. Hg.*	*% of control*	*liters/min.*	*kg. meters/min.*	*sec.*
2	F	15	—	—	—	2.15	0.14	0.65	4.3	1.2	130	—	0†	100	2.4	4.2	9.6
			3.9	50	5	—	5.7	0.45	—	—	115	—	+4†	10-14‡	2.7	4.2	13.3
			5.8	70	9	2.33	10.0	—	4.1	0.16	70	—	+5†	20-50‡	1.7	1.6	29.6
3	M	18	—	—	—	2.33	0.29	0.7	5.7	1.1	155	0	--	100	2.9	6.1	6.0
			0.9	3	17	—	4.2	—	—	(0.41)	155	+10	—	29-65‡	2.5	5.2	8.2
			2.4	10	12	2.35	6.8	0.5	4.8	0.23	130	+5	—	41-53‡	3.2	5.6	7.1
			3.7	15	14	—	9.2	—	—	—	58	+9	—	22-59‡	1.8	1.4	14.8
6	M	11	—	—	—	1.8	0.16	0.6	4.2	1.0	88	+1	—	100	2.4	2.9	5.3
			0.5	4	11	—	3.4	—	—	(0.38)	75	+3	—	73-80‡	1.7	1.7	7.4
			1.7	10	15	1.88	7.2	—	4.1	0.19	50	+14	—	33-67‡	0.89	0.6	20.9
10	M	20	—	—	—	1.93	0.20	—	6.5	0.83	93	-1	+5	100	—	—	—
			0.8	3	13	—	3.8	—	—	—	80	+6	+9	28-38‡	—	—	—
			1.5	7	9	—	5.5	—	—	—	75	+7	+10	31-38‡	—	—	—
11	M	17	—	—	—	2.48	0.32	—	6.2	1.10	103	+5	-3	100	1.6	2.2	6.9
			(0.4)	2	10	—	3.1	—	—	(0.54)	75	+9	-1	56	1.2	1.2	9.6
			3.0	19	10	2.48	7.0	—	5.3	0.25	70	+13	+3	42	1.0	1.0	15.3
			3.8	23	10	—	8.7	—	—	(0.20)	63	+13	+6	36	0.8	0.7	19.5
12	M	18	—	—	—	2.33	0.25	—	5.1	1.16	83	+4	+3	100	1.4	1.6	9.7
			(0.1)	1	5	—	2.0	—	—	—	55	+6	+4	50-57‡	1.0	0.8	13.3
			0.6	6	5	2.18	2.7	—	5.0	0.55	45	+6	+4	43	1.0	0.6	14.9

*Observations before infusion, at serum citrate levels comparable to those seen in patient experiments & at peak elevations. In dog No. 3 ventricular tachycardia followed by ventricular fibrillation occurred shortly after final determination. Hemodynamic changes in other 5 animals reversed by intravenously administered calcium chloride, epinephrine or acetylstrophanthidin (see text).

†Right atrium.

‡Pulsus alternans.

although serum citrate continued to rise.

Intravenous treatment with calcium chloride invariably reversed the circulatory effects of the citrate infusion in patients (M.H. and M.S., Table 1) or dogs (Fig. 2). Acetylstrophanthidin, given in doses of 200 to 300 microgm. to 2 animals, was also dramatically effective in reversing the hemodynamic effects of an elevated serum citrate. Epinephrine, infused at rates between 0.5 and 1.5 microgm. per kilogram of body weight per minute in 3 dogs, produced moderate to marked elevation in ventricular contractile force and blood pressure in 2, but in the third was associated with a sudden marked additional fall in blood pressure.

The present study demonstrates for the first time in man moderate to severe cardiac depression at elevations in serum citrate that have been reported to occur during rapid transfusions.[1-3] Circulatory depression occurred in man and in the dog at approximately the same blood levels, 2.5 to 4.0 millimols per liter (47 to 75 mg. per 100 ml.). Critical circulatory depression or death occurred in the dog at blood levels of 2.7 to 10 millimols per liter (50 to 190 mg. per 100 ml.) and at rates of infusion of citrate of 10 to 15 mg. per kilogram of body weight per minute — very close to the fatal dose rates reported in early studies.[19,20]

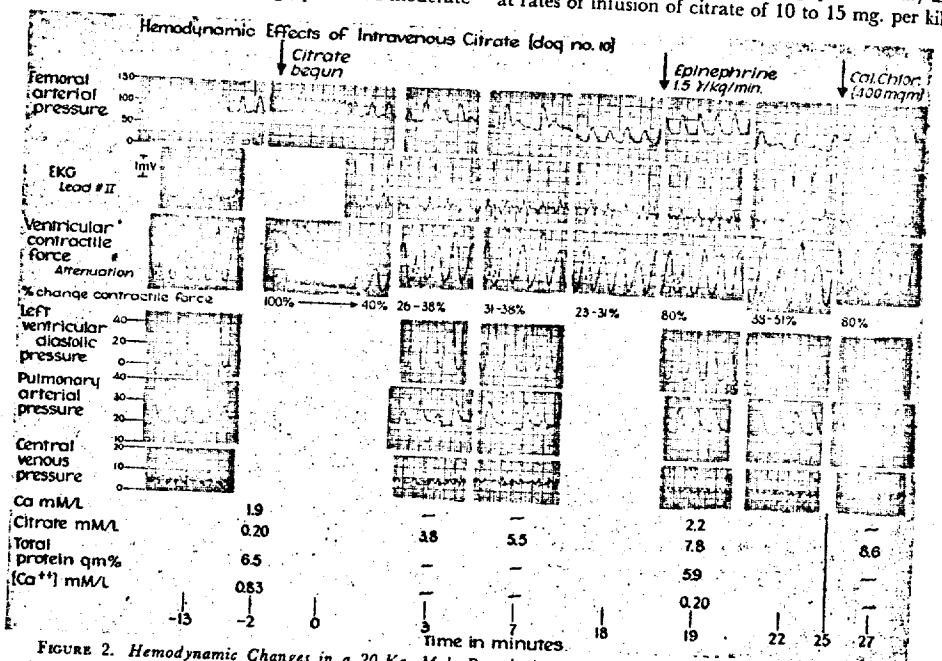

FIGURE 2. *Hemodynamic Changes in a 20-Kg. Male Dog during Infusion of 10 per Cent Sodium Citrate. Note the marked fall in the contractile force of the left ventricle at the beginning of the citrate infusion and at a time when arterial-blood pressure is well maintained. Note also the early development of pulsus alternans and the virtual disappearance of alternate pulse waves after seven minutes. T waves are depressed in the electrocardiogram at the onset of the citrate infusion. Epinephrine briefly corrects hypotension and other cardiovascular disturbances, but pressure and contractile force fall again in spite of continued epinephrine infusion. Calcium chloride markedly improves all disturbances, including depressed T waves of the electrocardiogram.*

DISCUSSION

The possible harmful effects of elevations of serum citrate during transfusion of citrated blood presumably are solely secondary to the binding of calcium, with resultant hypocalcemia. The theoretical possibility of such "citric acid intoxication" has long been recognized but often discounted.[2,4] Electrocardiographic evidence of hypocalcemia during transfusion of citrated blood has previously been reported.[2,17,18]

Circulatory depression in man and the dog was manifested by hypotension, by variable changes in cardiac output and by narrowed pulse pressure, as well as by electrocardiographic evidence of hypocalcemia. In addition direct evidence of cardiac depression was provided in the dog by the strain-gauge measurements and supported by the rise in central venous pressure and end-diastolic pressure in the left ventricle. Such an increase in filling pressure would lead in the not-depressed heart to a substantial increase in cardiac output. Cardiac output, which was variable in response (but usually fell), certainly underestimates the

existing depression because of the simultaneous fall in arterial pressure. The calculated work of the left ventricle more accurately reflects the existing cardiac depression, and, in fact, this value was decreased in every experiment.

The well known effect of calcium given intravenously (in these studies as the chloride salt) in preventing and correcting the circulatory depression of citrate intoxication was readily demonstrated. Antagonism of the circulatory effects of hypocalcemia by digitalis or related compounds, demonstrated in 2 of the animal experiments of this study, has previously been reported.[21] The partial protective effect of intravenously administered epinephrine and the markedly increased sensitivity of the sympathetic-blocked dog to the depressant effects of intravenously given sodium citrate suggest that endogenous epinephrine may be involved in the protection against hypocalcemia during rapid transfusion.

To achieve elevations in serum citrate at which hemodynamic evidence of hypocalcemia is demonstrated in the present study, it would be necessary to infuse multiple units of citrated blood at a very rapid rate — for example, as fast as 1 unit (500 ml.) every three or four minutes to a patient weighing 70 kg. It is not unusual in wartime or in civilian disasters to transfuse at such speeds, but it is certainly less common in routine clinical practice. However, it is likely that tolerance to an elevated serum citrate and hypocalcemia is less in the debilitated patient than in the healthy young patients studied. For example, it may be supposed that the patient with prior poor cardiac function[22] or the patient in hemorrhagic shock[23] may exhibit considerably less tolerance. It also seems likely that in the complex clinical situation in which multiple rapid transfusions are necessary the effects of other factors such as hyperkalemia,[24] increased hydrogen ion concentration[25,26] and anesthetic[28] may share with citric acid an additive effect on the etiology of circulatory disaster.

Summary

The hemodynamic effects of sodium citrate given intravenously were studied in 6 patients and in 9 dogs. At concentrations of serum citrate observed by us and by others to occur during multiple rapid infusions of citrated blood, moderate to marked circulatory depression was noted: hypotension; depression of pulse pressure; variable fall in cardiac output; depression of the work of the left ventricle; and electrocardiographic evidence of hypocalcemia in man. Similar changes were observed in the dog, and in addition, marked depression of ventricular contractile force and moderate to marked elevations of ventricular end-diastolic and central venous pressures. Calcium chloride given intravenously was uniformly effective in correcting these changes.

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(German Publication pp. 128-130)

Contribution to the flame-photometric CaO determination in feeds and soils

(From the Oldenburg Agricultural Research Office and Testing Station of the Weser-Ems Chamber of Agriculture; Director: F. Nieschlag)

By H. Westerhoff (*)

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Phosphates, iron ions and especially aluminum ions interfere considerably with the flame-photometric determination of calcium. In our own experience, solutions containing 10 mg of Al besides 50 mg of CaO in 100 ml were found to show CaO deficits of 25%. In testing feeds and soils, deficits up to 35% were sometimes found. Several authors have tried to eliminate these errors but have not attained practicable results (1,2). In the paper of Gjems and Lädersen (2) the attempt is made to eliminate these disturbances in the CaO determination by precipitating the aluminum by means of aluminum benzoate. This, however, is possible only in cases where very little P_2O_5 is present. For feeds this method furnishes no usable results. According to the method developed by Schneider (3), the CaO content is determined after addition of ammonium citrate and separation of the P_2O_5 with magnesia mixture. But the deviations from the gravimetrically determined values are still relatively high.

As flame photometer we used a converted old Schuhknecht-Waibel instrument, in which the selenium cell S_{28} of the firm Lange had been installed as photoelement. The flame was produced by an acetylene-air mixture. As light filter we used the KR_1 filter of Musterschmidt, Göttingen. But as this filter showed a certain transmissivity for lines caused by potash compounds, it was necessary to correct the galvanometer deflections measured by CaO in the presence of potash. It was found, however, that under the given operating conditions these corrections were independent of the CaO quantity and pro-

portional to the quantity of potash present. While it is necessary, when using this light filter, to determine the quantity of potash present, this can be done very easily by flame photometry. It is probably very easy to eliminate this circumstance by using interference or other suitable light filters.

The disturbance of the determination by the presence of phosphoric acid is dependent on the P_2O_5 and CaO content of the solutions to be examined. Köhnlein and Lücke (1) noted that with increasing addition of P_2O_5 to constant CaO quantities the galvanometer deflections decreased only up to a certain phosphate concentration (CaO : P_2O_5 = 1.23), then remaining constant at higher P_2O_5 quantities. One can utilize this finding in Ca determination by adding to the solution to be tested enough phosphate from the start so that this CaO : P_2O_5 ratio is exceeded even at the highest CaO quantity to be expected. For this reason I have added to each CaO determination 100 mg P_2O_5 as $(NH_4)_2HPO_4$ or $NH_4H_2PO_4$.

It is much more difficult to eliminate the disturbance caused by iron and aluminum ions. Addition of tartrate or citrate ions, to form an iron and aluminum complex, was not successful. The best way proved to be to precipitate the sesquioxides by means of ammonium acetate and to determine the CaO content in the filtrate by flame photometry. The best procedure is to admix an aliquot part of the hydrochloric acid ash solution, which should contain 1 ml of 25% HCl, with 2 ml of phosphate solution (100 mg P_2O_5), diluting with water in a 100-ml flask to about 70 ml, and bringing to a boil. Then one adds 4 ml of 50% ammonium acetate solution, again letting boil briefly. After cooling, filter through a pleated filter and atomize the resulting filtrate in the flame photometer. The ammonium acetate should be added to the boiling solution, and a filtration must be carried out also when no visible precipitate has formed. The standard solutions for calibrating the flame photometer are prepared by adding 1 ml of 25% HCl, 2 ml of ammonium phosphate solution,

and 4 ml of ammonium acetate solution in a 100-ml flask to a solution of 1, 2 .... 10 mg CaO, and filling up with water to 100 ml.

That this relatively simple method furnishes useful values for the Ca content of feeds will be evident from the following table. In 41 feed samples the CaO quantities obtained by precipitation with ammonium oxalate were compared with those determined by flame photometry. It was found that in 54% of all samples the deviation of the CaO contents determined by flame photometry from the values determined with ammonium oxalate was below 5%. In approximately one third of the samples errors between 5 and 10% were found. In 14.5% of the tests the error was somewhat greater than 10%.

For the 8 peat soils tested, the determined deviations, insofar as these few findings permit a conclusion at all, were somewhat greater. But they range in approximately the same order of magnitude (see table alongside).

Sample #	Designation	% CaO m.NH_4-oxalate	Flame photometry	% error relative	Absolute
200	Hay	2.60	2.63	+0.03	+1.1
2072	Silage	2.00	1.97	-0.03	-1.5
2213	"	1.74	1.70	-0.04	-2.3
2214	"	2.17	2.03	-0.14	-8.2
2221	"	0.81	0.75	-0.05	-6.1
2379	Carrots	0.38	0.40	+0.02	+5.3
1919	Silage	2.00	1.81	-0.19	-9.5
2420	"	1.10	0.98	-0.12	-10.9
2421	"	1.31	1.34	+0.03	+2.3
2422	"	1.31	1.27	-0.04	-3.0
2423	"	0.97	1.00	+0.03	+3.1
2810	Stinging nettle	1.64	1.56	-0.08	-4.9
2827	Hay	1.23	1.24	+0.01	+0.8
2828	Grass	0.84	0.92	+0.08	+9.5
2837	Dry Green fodder	0.76	0.76	0	0
2839	"	0.63	0.70	+0.07	+11.1
2842	Young grass	0.98	1.05	+0.07	+7.2
2863	Landsb. Mix	1.45	1.60	+0.15	+10.3
2864	Dry green fodder	0.70	0.80	+0.10	+14.3
2865	"	0.97	1.05	+0.08	+8.3
2881	Green meal	1.07	1.08	+0.01	+0.9
2882	"	0.75	0.78	+0.03	+4.0
2883	"	0.70	0.73	+0.03	+4.3

2884	Green meal	0.69	0.78	+0.09	+13.1
2885	"	0.75	0.80	+0.05	+6.7
1996	Dry chips	1.40	1.34	-0.06	-4.2
1997	Troblaco	1.21	1.14	-0.07	-5.8
1998	Hay	0.89	0.86	-0.03	-3.4
1999	"	1.16	1.12	-0.04	-3.4
2001	"	2.60	2.52	-0.08	-3.1
2002	Pea straw	1.50	1.48	-0.02	-1.3
2525	Silage	0.41	0.40	-0.01	-2.4
2543	Grass	0.83	0.76	-0.07	-8.4
1976	Silage	2.10	2.25	+0.15	+7.1
1977	Troblaco	1.93	1.81	-0.12	-6.4
2335	Silage	1.26	1.27	+0.01	+0.8
2434	Wheat bran	0.75	0.72	-0.03	-4.0
2436	Soy grit	0.46	0.46	0	0
2437	Peanut grit	0.26	0.25	-0.01	-3.8
2438	Palm kernel grit	0.52	0.58	+0.06	+11.5
2505	Meadow grass	1.71	1.60	-0.09	-6.4
1036	Peat	0.66	0.73	+0.07	+10.6
745	"	2.27	1.92	-0.29	-15.4
746	"	2.82	2.52	-0.30	-10.7
747	"	2.12	2.07	-0.05	-2.4
748	"	1.96	1.84	-0.12	-6.1
749	"	1.89	1.88	-0.01	-0.5
750	"	2.28	2.10	-0.18	-7.9
751	"	4.24	3.92	-0.32	-7.5

Method

Solutions required:

1. CaO: 1 g CaO for analysis is dissolved in little HCl and filled up to 1 liter with H_2O, 1 ml = 1 mg CaO.

2. 500 g of NH_4 acetate are dissolved in water to make 1 liter.

3. Dissolve 92 g $(NH_4)_2HPO_4$ to 1 liter water.

2.5 g of substance are reduced to ash, transferred to a 100-ml flask, added with 10 ml of 25% HCl, and heated to boiling. After cooling, fill up to the mark. Let the undissolved parts settle briefly and transfer 10 ml of the solution into a 100-ml flask with a pipette, dilate with H_2O to approximately 70 ml, add 2 ml $(NH_4)_2HPO_4$ solution and heat to boiling on a wire mesh. To the boiling hot solution add 4 ml NH_4 acetate solution, boil again briefly, let cool, fill up to the mark, and filter through a pleated filter. The solution thus obtained is atomized in the flame photometer.

Standard solutions: To 0.5; 1 .... 10 mg of CaO in 100-ml flasks there are

added 1 ml of 25% HCl, 2 ml of $(NH_4)_2HPO_4$ solution and 4 ml of NH_4-acetate solution, filling up to 100 ml.

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Beitrag zur flammenphotometrischen CaO-Bestimmung in Futtermitteln und Böden

(Aus dem Landw. Untersuchungsamt u. Versuchsanstalt Oldenburg der Landwirtschaftskammer Weser-Ems; Direktor: F. Nieschlag)

Von H. Westerhoff[1])

(Eingegangen am 20. 10. 1954)

Phosphate, Eisen- und besonders Aluminiumionen stören die flammenphotometrische Bestimmung des Calciums sehr erheblich. Nach eigenen Erfahrungen wurden in Lösungen, die in 100 ml neben 50 mg CaO noch 10 mg Al enthielten, Mindergehalte an CaO von 25% festgestellt. Bei der Untersuchung von Futtermitteln und Böden ergaben sich teilweise Mindergehalte bis 35% CaO. Mehrere Autoren haben sich bemüht, diese Fehler auszuschalten, ohne jedoch zu praktisch brauchbaren Ergebnissen zu gelangen (1, 2). In der Arbeit von Gjems und Lüdersen (2) wird versucht, diese Störungen der CaO-Bestimmung durch Ausfällen des Aluminiums mit Hilfe von Aluminiumbenzoat zu beseitigen. Dies gelingt aber nur in Fällen, in denen sehr wenig P_2O_5 anwesend ist. Bei Futtermitteln liefert diese Methode keine brauchbaren Ergebnisse. Nach der von Schneider (3) ausgearbeiteten Methode wird der CaO-Gehalt nach Zusatz von Ammonzitrat und Abscheidung der P_2O_5 mit Magnesiamischung vorgenommen. Die Abweichungen von den gravimetrisch ermittelten Werten sind aber noch verhältnismäßig hoch.

Als Flammenphotometer diente ein umgebautes altes Schuhknecht-Waibelgerät, in welches als Photoelement die Selenzelle S_{25} der Firma Lange eingebaut worden war. Die Flamme wurde durch Acetylen/Luftgemisch erzeugt. Als Lichtfilter wurde das Filter KR_1 der Firma Musterschmidt, Göttingen, verwendet. Da dieses Filter aber eine gewisse Durchlässigkeit für durch Kaliverbindungen erzeugte Linien zeigte, mußten die bei Anwesenheit von Kali durch CaO gemessenen Galvanometerausschläge korrigiert werden. Es zeigte sich aber, daß diese Korrekturen unter den gegebenen Arbeitsbedingungen unabhängig von der CaO-Menge und der vorhandenen Kalimenge proportional waren. Es ist bei Verwendung dieses Lichtfilters zwar erforderlich, die vorhandene Kalimenge zu ermitteln, doch läßt sich dies flammenphotometrisch sehr leicht durchführen. Durch Verwendung von Interferenz- oder sonstigen geeigneten Lichtfiltern läßt sich dieser Umstand aber wahrscheinlich leicht beseitigen.

Die Störung der Bestimmung durch vorhandene Phosphorsäure ist abhängig von dem P_2O_5- und dem CaO-Gehalt der zu untersuchenden Lösungen. Köhnlein u. Lücke (1) haben festgestellt, daß bei steigendem Zusatz von P_2O_5 zu konstanten CaO-Mengen die Galvanometerausschläge nur bis zu einer bestimmten Phosphatkonzentration (CaO : P_2O_5 = 1,23) abnahmen, um dann bei höheren P_2O_5-Mengen konstant zu bleiben. Diesen Befund kann man sich nun bei der Ca-Bestimmung insofern zunutze machen, als man von vornherein der zu untersuchenden Lösung soviel Phosphat zufügt, daß dieses CaO : P_2O_5-Verhältnis auch bei der höchsten zu erwartenden CaO-Menge überschritten wird. Ich habe deshalb zu jeder CaO-Bestimmung 100 mg P_2O_5 als $(NH_4)_2HPO_4$ oder $NH_4H_2PO_4$ zugefügt.

Weit schwieriger ist es, die durch Eisen- und Aluminiumionen verursachte Störung auszuschalten. Zusatz von Tartrat- oder Zitrationen, um Eisen und Aluminium komplex zu binden, führten nicht zum Ziele. Als am besten geeignet erwies es sich, die Sesquioxyde mit Hilfe von Ammoniumacetat auszufällen und im Filtrat den CaO-Gehalt flammenphotometrisch zu ermitteln. Man verfährt dabei am besten so, daß man einen aliquoten Teil der salzsauren Aschenlösung, der 1 ml 25%ige HCl enthalten soll, mit 2 ml Phosphatlösung (100 mg P_2O_5) versetzt, in einem 100 ml Kolben auf ungefähr 70 ml mit Wasser verdünnt und zum Sieden erhitzt. Dann fügt man 4 ml 50%ige Ammonacetatlösung hinzu und läßt nochmals kurz aufkochen. Nach dem Abkühlen filtriert man durch ein Faltenfilter und zerstäubt das erhaltene Filtrat im Flammenphotometer. Es ist dabei darauf zu achten, daß das Ammonacetat zur siedenden Lösung gegeben wird und daß eine Filtration auch dann vorgenommen werden muß, wenn sich kein sichtbarer Niederschlag gebildet hat. Die Standardlösungen zur Eichung des Flammenphotometers werden hergestellt, indem man in einem 100-ml-Kölbchen zu einer Lösung von 1, 2 .... 10 mg CaO, 1 ml 25% HCl, 2 ml Ammonphosphatlösung und 4 ml Ammonacetatlösung hinzugibt und auf 100 ml mit Wasser auffüllt.

Daß diese verhältnismäßig einfache Methode brauchbare Werte für den Ca-Gehalt von Futtermitteln ergibt, wird durch die Befunde der folgenden Tabelle erhärtet. Es wurden bei 41 Futtermittelproben die durch Fällen mit Ammonoxalat erhaltenen CaO-Mengen mit den flammenphotometrisch ermittelten verglichen. Es zeigte sich dabei, daß bei 54% aller Proben die Abweichung der flammenphotometrisch ermittelten CaO-Gehalte von den mit Ammonoxalat ermittelten Werten unter 5% lag. Bei ungefähr einem Drittel der Proben ergaben sich Fehler zwischen 5 und 10%. Bei 14,5% der Untersuchungen war der Fehler etwas größer als 10%.

Bei den 8 untersuchten Moorböden sind die ermittelten Abweichungen, sofern diese wenigen Befunde überhaupt einen Schluß erlauben, zwar etwas größer. Sie bewegen sich aber in ungefähr derselben Größenordnung (siehe nebenstehende Tabelle).

Nr. der Probe	Bezeichnung	% CaO m. NH_4-oxalat	% CaO flammenphotometrisch	% Fehler relativ	% Fehler absolut
2000	Heu	2,60	2,63	+0,03	+ 1,1
2072	Silofutter	2,00	1,97	−0,03	− 1,5
2213	.	1,74	1,70	−0,04	− 2,3
2214	.	2,17	2,03	−0,14	− 8,2
2221	.	0,81	0,76	−0,05	− 6,1
2379	G. K. Rüben	0,38	0,40	+0,02	+ 5,3
1919	Silofutter	2,00	1,81	−0,19	− 9,5
2420	.	1,10	0,98	−0,12	−10,9
2421	.	1,31	1,34	+0,03	+ 2,3
2422	.	1,31	1,27	−0,04	− 3,0
2423	.	0,97	1,00	+0,03	+ 3,1
2810	Brennessel	1,64	1,56	−0,08	− 4,9
2827	Heu	1,23	1,24	+0,01	+ 0,8
2828	Gras	0,84	0,92	+0,08	+ 9,5
2837	Trockengrünfutter	0,76	0,76	0	0
2839	.	0,63	0,70	+0,07	+11,1
2842	Junggras	0,98	1,05	+0,07	+ 7,2
2863	Landsb. Gemenge	1,45	1,60	+0,15	+10,3
2864	Trockengrünfutter	0,70	0,80	+0,10	+14,3
2865	.	0,97	1,05	+0,08	+ 8,3
2881	Grünmehl	1,07	1,08	+0,01	+ 0,9
2882	.	0,75	0,78	+0,03	+ 4,0
2883	.	0,70	0,73	+0,03	+ 4,3
2884	.	0,69	0,78	+0,09	+13,1
2885	.	0,75	0,80	+0,05	+ 6,7
1996	Trockenschnitzel	1,40	1,34	−0,06	− 4,2
1997	Troblaco	1,21	1,14	−0,07	− 5,8
1998	Heu	0,89	0,86	−0,03	− 3,4
1999	.	1,16	1,12	−0,04	− 3,4
2001	.	2,60	2,52	−0,08	− 3,1
2002	Erbsenstroh	1,50	1,48	−0,02	− 1,3
2525	Silofutter	0,41	0,40	−0,01	− 2,4
2543	Gras	0,83	0,76	−0,07	− 8,4
1976	Silofutter	2,10	2,25	+0,15	+ 7,1
1977	Troblaco	1,93	1,81	−0,12	− 6,4
2335	Silage	1,26	1,27	+0,01	+ 0,8
2434	Weizenkleie	0,75	0,72	−0,03	− 4,0
2436	Sojaschrot	0,46	0,46	0	0
2437	Erdnußschrot	0,26	0,25	−0,01	− 3,8
2438	Palmkernschrot	0,52	0,58	+0,06	+11,5
2505	Knaulgras	1,71	1,60	−0,09	− 6,4
1036	Moorboden	0,66	0,73	+0,07	+10,6
745	.	2,27	1,92	−0,29	−15,4
746	.	2,82	2,52	−0,30	−10,7
747	.	2,12	2,07	−0,05	− 2,4
748	.	1,96	1,84	−0,12	− 6,1
749	.	1,89	1,88	−0,01	− 0,5
750	.	2,28	2,10	−0,18	− 7,9
751	.	4,24	3,92	−0,32	− 7,5

Durchführung der Bestimmung:

Erforderliche Lösungen:

1. CaO : 1 g CaO zur Analyse wird in wenig HCl gelöst und zu einem l mit H_2O aufgefüllt, 1 ml = 1 mg CaO.

2. 500 g NH_4acetat werden zu 1 l in Wasser gelöst.

3. 92 g $(NH_4)_2HPO_4$ zu 1 l Wasser lösen.

2,5 g Substanz werden verascht, in ein 100-ml-Kölbchen übergespült, mit 10 ml 25%iger HCl versetzt und zum Sieden erhitzt. Nach dem Abkühlen wird bis zur Marke aufgefüllt. Man läßt die ungelösten Teile kurz absitzen und pipettiert 10 ml der Lösung in ein 100-ml-Kölbchen, verdünnt mit H_2O auf ung[illegible] 70 ml, fügt 2 ml $(NH_4)_2HPO_4$-Lösung hinzu [illegible]zt auf dem Drahtnetz zum Sieden. Zur siedendheißen Lösung gibt man 4 ml NH_4-Acetatlösung, läßt nochmals

[1]) Der landw.-techn. Assistentin Frl. A. Reiners bin ich für die rege Mitarbeit bei der praktischen Durchführung der Versuche sehr dankbar.

kurz aufkochen, kühlt ab, füllt zur Marke auf und filtriert durch ein Faltenfilter. Die so erhaltene Lösung wird im Flammenphotometer zerstäubt.

Standardlösungen: Zu 0,5; 1 .... 10 mg CaO in 100-ml-Kölbchen werden 1 ml 25%ige HCl, 2 ml $(NH_4)_2HPO_4$-Lösung und 4 ml NH_4-acetatlösung gegeben und auf 100 ml aufgefüllt.

Schrifttum

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Karotingehalt und Heuqualität

(Aus dem Staatl. Forschungs- und Beratungsinstitut für Höhenlandwirtschaft, Donaueschingen, Direktor: Prof. Dr. Knoll)

Von K. Papendick

(Eingegangen am 25. 9. 1954)

Eine wesentliche Karotinquelle ist das Heu, das zu den wichtigsten wirtschaftseigenen Futterstoffen gehört und die Grundlage einer normalen Winterfütterung im Viehstall bildet. Über den Karotingehalt von Wiesenheu liegen bisher verhältnismäßig wenige Ergebnisse vor, so daß es wünschenswert erschien, den Karotingehalt des Wiesenheues unter den örtlichen Bedingungen des Ostschwarzwaldes zu prüfen. Da das Karotin ein sehr empfindlicher Bestandteil der Nahrung ist, wurde außerdem die Frage untersucht, inwieweit Beziehungen zwischen dem Karotingehalt des Heues und seinem Gehalt an verdaulichen Nährstoffen bestehen, d. h. was der Karotingehalt über die Güte des Heues aussagen kann.

Unter Karotin versteht man heute die isolierbaren Vitamin-A-wirksamen Karotinoide α-, β- und γ-Karotin und Kryptoxanthin. In den üblichen Futterstoffen kommt hauptsächlich das β-Karotin vor. Karotin wirkt nicht selbst als Vitamin, sondern stellt die Vorstufe des in der Ernährung der Tiere so wichtigen Vitamins A dar. Aus den genannten Karotinoiden wird im Tierkörper, teils in der Leber, teils schon im Darm das Vitamin A gebildet. Dabei können aus α-Karotin, γ-Karotin und Kryptoxanthin je ein Teil Vitamin A und aus β-Karotin 2 Teile Vitamin A entstehen. Bei der Umwandlung des Karotins treten jedoch Verluste auf, weswegen die theoretisch mögliche Vitamin-A-Ausbeute praktisch niemals erreicht wird. Über die Größe der Umwandlungsverluste lassen sich noch keine allgemeinen quantitativen Aussagen machen. Deshalb betragen die für die Praxis angegebenen Bedarfsnormen im allgemeinen ein Vielfaches des physiologischen Minimums und schwanken nicht unerheblich.

Da alle grünen Pflanzen und die daraus gewonnenen Futterstoffe, also Grünfutter, Gärfutter, Heu, Grünmehle usw., kein Vitamin A, sondern nur die Vorstufe Karotin enthalten, ist das Karotin die einzige Vitamin-A-Quelle bei den Pflanzenfressern. Große Bedeutung kommt daher dem Karotingehalt des Futters besonders in der Milchviehfütterung zu.

Das Karotin ist wenig widerstandsfähig gegen äußere Einflüsse und erleidet mehr oder weniger starke Einbußen. Mit der Karotinzersetzung gehen die Verluste an anderen Bestandteilen des Futters einher. Da das Karotin jedoch wesentlich empfindlicher ist als die übrigen Nährstoffe, war zu vermuten, daß der Karotingehalt zur Beurteilung der Rauhfutterqualität, insbesondere auch des Heues, herangezogen werden kann. Der absolute Gehalt an Karotin hängt natürlich von verschiedenen Faktoren wie der Pflanzenart, der Schnittzeitpunkt, der Konservierung, der Lagerung usw. ab. Man muß grundsätzlich unterscheiden zwischen Grünfutter und dem daraus gewonnenen natürlichen Heu. Wiesengras hat in 1 kg Trockensubstanz durchschnittlich 200 bis 300 mg Karotin, Wiesenheu nur noch durchschnittlich 10 bis 20 mg, d. h. bei der Heuwerbung gehen bis zu 90% und mehr d

XXXVII - TREATMENT OF LYMPHANGIOMAS BY SCLEROSING INJECTIONS OF SODIUM CITRATE

by Fonseca E. Castro, Professor of Pediatrics, Faculty of Medicine of Porto (Portugal)

Director: Prof. A. Garrett

The treatment of angiomas and lymphangiomas by modifying injections is not new. But the tests made a little everywhere were not capable of determining a method, either because of technical difficulties (the injections often produced necroses) or because of insufficient results.

The use of coagulating substances was quickly abandoned. The use of sclerosing substances offered much greater interest. These substances, large in numbers, were used in different manners by many authors.

Some of these fell into disuse after a short time; thus tincture of iodine, essence of turpentine (Suner), bi-iodide of mercury (Montpellier and Lacroix), salts of magnesium (Kato and Gerlach). Others were more widely used: sodium salicylate (Tytgat, Touraine and Renault, etc.), quinine-urethane or quinine-urea (Fildermann, Etienne, Le Blaye, etc.), and finally sodium citrate (Hustin).

Of all these substances sodium citrate causes the least irritation. Injected into the angiomas, the other substances easily produce eschars, if these solutions are not carefully introduced inside the blood pools. Only citrate, possessing the same sclerosing properties, is almost inoffensive, for it has no necrosing action if the quantities injected are reasonable. This is why Sicard preferred it for the treatment of varicose veins and J. Troisier uses it in the therapy of certain arterites. In our hands it proved a very satisfactory means to obtain the reduction, by sclerosis, of tuberous angiomas; we adjusted this process, as regards its technique and its indications, on the basis of experience gained through dozens of observations (1).

We use the saturated solution of sodium citrate (at approximately 60%) which we inject into the depth of the tumor; if the latter is voluminous, we perform 2 or 3 injections at different points; each injection must not introduce more than 1 cc, and they are generally smaller (0.5 cc). In order to repeat the in-

jections the reaction caused by the previous ones must have disappeared since several days; in this manner the treatment is performed over several days or several weeks, which makes this method very useful in rural clinical work.

We applied these technical prescriptions for the treatment of angiomas by injections of sodium citrate in some cases of lymphangiomas and we believe that, in these cases, the method had not yet been used. The results are still better than those obtained in most cases of angiomas. Although we have only three observations of lymphangioma treated with sodium citrate, the constancy of healing and the uniformity of the regressive evolution of the tumor indicate that we are in possession of an excellent therapeutical process.

Following is a summary of these three cases:

I. M. C., aged 15 days, is seen for the first time (Hôpital de Saint-Antoine) on February 25, 1929; she displays a lymphangioma in the upper right clavicular region, having the volume of a small orange (Fig. 1). This same day we begin the treatment by injections into the tumor of sodium citrate solution (2 cc); this therapy is continued with injections every four days, for two months. At the end of this period the lymphangioma is completely reduced (Fig. 2).

Today, nine years later, this tumor has disappeared without sign.

II. L.F.S., aged 18 months, is registered in the consultation service of Hôpital Saint-Antoine) for a voluminous lymphangioma of the right axillary region (Fig. 3) which existed already at birth. On May 2 this infant receives the first citrate injections (2 cc) in the tumor; the injections are repeated at intervals of 3 to 5 days. After the 4th injection one observes a hardening of the tumoral mass, with a slight inflammatory reaction which persists for one week; then there is a gradual regression of the lymphangioma and the treatment is resumed; the patient receives 8 more injections with smaller doses (1 to 1-1/2 cc) at greater intervals (in addition, an injection of 1 cc of 20% solution of sodium salicylate). He was then much improved; the tumor produced only a small tumefaction, of a pasty consistency, in the middle of which small nodes could be felt by palpation.

Later, after approximately one year, the reduction was even more definite and the nodes had disappeared (Fig. 4); only a small infiltration remains, barely

recognizable by palpation, and with the arm in adduction.

III. M.C.S., aged 20 days, registered for consulation (Hôpital de Saint-Antoine) on March 3, 1936, for a large congenital lymphangioma. It resides in the left cervical region, occupying a large space having the following limits; in front, a line descending fromthe parotidian region down to the chin, and hence up to the sternal manubrium, following the clavicle, around the scapular region and, following the nape of the neck, rising back to the ear pavillion (Fig. 5). This tumor, covered with fine skin, without arborizations, is slightly lobed by two shallow vertical furrows; it contains a serous liquid which issues easily by puncture.

After the first citrate injection (3 cc) it seems that a reduction already begins to take place; it becomes definite after the second injection, given two weeks later, and for this reason the third injection is only 1 cc. The tumor then had the volume of a tangerine. The tendency to healing was so remarkable that no further treatment was performed. Less than two months had passed after the first injection that the tumor was reduced to a small nodule, barely perceptible under palpation (Fig. 6). Today (July 1938) only the folds of the skin betray the previous existence of the tumoral mass (Fig. 7).

Such, in their simplicity, are our three observations. They show that sodium citrate, in saturated solution and suitably injected, reduces lymphangiomas to such an extent that they can be considered as healed.

Healing comes after a variable number of injections, which was very small (only 3) in our third observation; in this patient the regression was so extraordinarily precocious that we believe we have fallen upon a case (certain rare) with tendency to spontaneous sclerosis; but, even in these conditions, the effectiveness of the therapeutic method leaves no doubt.

The reduction of the lymphangioma corresponds to a true healing, as demonstrated by the stability of the results after a few years.

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XXXVII

TRAITEMENT DES LYMPHANGIOMES PAR DES INJECTIONS SCLÉROSANTES DE CITRATE DE SOUDE

par FONSECA E CASTRO
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Directeur : Prof. A. GARRETT

Le traitement des angiomes et des lymphangiomes par des injections modificatrices n'est pas nouveau. Mais les essais réalisés un peu partout n'avaient réussi à imposer la méthode, soit par des difficultés de technique (les injections étaient souvent nécrosantes), soit par l'insuffisance des résultats.

L'emploi de substances coagulantes fut vite abandonné. Celui des substances sclérosantes a beaucoup plus d'intérêt. Ces substances, en grand nombre, ont été utilisées de diverses façons par plusieurs auteurs.

Quelques-unes sont tombées en désuétude à bref délai ; ainsi la teinture d'iode, l'essence de térébenthine (Suñer), le bi-iodure de mercure (Montpellier et Lacroix), les sels de magnésium (Kato et Gerlach). D'autres ont été plus largement employées : le salicylate de soude (Tytgat, Touraine et Renault, etc.), le quinine-uréthane ou le quinine-urée (Fildermann, Etienne, Le Blaye, etc.), et enfin le citrate de soude (Hustin).

De toutes ces substances, c'est le citrate de soude qui provoque moins d'irritation. Injectées dans les angiomes, les autres substances produisent facilement des eschares, si les solutions ne sont pas introduites soigneusement dans l'intérieur des lacs sanguins. Seul le citrate, doué des mêmes propriétés sclérosantes, est presque inoffensif, car il n'a pas d'action nécrosante si les quantités injectées sont raisonnables. C'est pour cela que Sicard l'a préféré pour le traitement des varices et J. Troisier l'utilise dans la thérapeutique de certaines artérites. Il s'est montré, dans nos mains, un moyen

FIG. 1

FIG. 2

FIG. 3

FIG. 4

Sodium Citrate 56

56

très satisfaisant pour obtenir la réduction, par la sclérose, des angiomes tubéreux ; ce procédé, nous l'avons réglé, dans sa technique et ses indications, sur l'expérience de plusieurs dizaines d'observations (1).

Nous employons la solution saturée de citrate de soude (environ à 60 %), que nous injectons dans la profondeur de la tumeur ; si celle-ci est volumineuse, nous faisons 2 ou 3 piqûres, en des points divers ; chaque piqûre ne doit pas injecter plus de 1 cc., et généralement elles sont plus petites (0,5 cc.). Pour renouveler les injections, il faut que la réaction qu'elles provoquent soit entièrement disparue depuis quelques jours ; de cette façon, le traitement

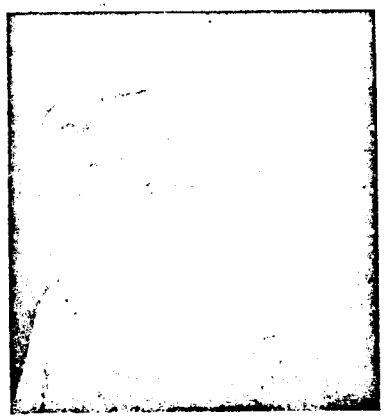

Fig. 5

se fait de jours en jours ou de semaines en semaines, ce qui rend la méthode très utile dans la clinique rurale.

Ces prescriptions techniques du traitement des angiomes par les injections de citrate de soude, nous les avons appliquées dans quelques cas de lymphangiomes, et nous croyons que, dans ces cas, la méthode n'avait pas encore été employée. Les résultats sont encore supérieurs à ceux obtenus dans la plupart des cas d'angiomes. Bien que nous n'ayons que trois observations de lymphangiomes traités par le citrate de soude, la constance de la guérison et l'uniformité de l'évolution régressive de la tumeur laissent prévoir que nous sommes en possession d'un excellent procédé thérapeutique.

(1) Fonseca e Castro et Macedo Chaves. — Tratamento dos angiomas e linfangiomas por injecções locais (*Portugal Médico*, n° 8 de 1929).
Fonseca e Castro et Macedo Chaves. — Notas sôbre o tratamento dos angiomas e linfangiomas por injecções esclerosantes (*Portugal Médico*, n° 3 de 1932).

Voici, en résumé, ces trois cas :

I. — M. C., âgée de quinze jours, est vue pour la première fois (Hôpital de Saint-Antoine) le 25 février 1929 ; elle présente un lymphangiome dans la région susclaviculaire droite, ayant le volume d'une petite orange (Fig. 1). Ce même jour commence le traitement par des injections, dans la tumeur, de solution de citrate de soude (2 cc.); on poursuit cette thérapeutique, avec des injections de 4 en 4 jours, pendant deux mois. A la fin de cette période, le lymphangiome est complètement réduit (Fig. 2).

Aujourd'hui, neuf années après, on ne voit pas de signe de la tumeur disparue.

II. — L. F. S., âgé de 18 mois, est inscrit dans le service de consultations de l'Hôpital de Saint-Antoine pour un volumineux lymphangiome

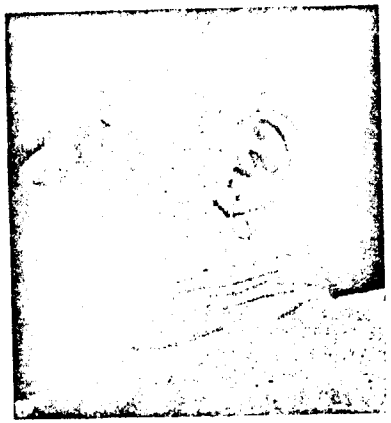

Fig. 6

de la région axillaire droite (Fig. 3), qui existait déjà au moment de la naissance. Le 2 mai, ce petit reçoit dans la tumeur les premières injections de citrate (2 cc.) ; les injections se répètent avec intervalles de 3 à 5 jours. Après la quatrième injection on note l'endurcissement de la masse tumorale, avec légère réaction inflammatoire, qui persiste pendant une semaine ; puis, on assiste à la régression progressive du lymphangiome et on reprend le traitement ; il reçoit encore 8 injections, avec des doses plus petites (1 à 1 cc. 1/2), plus distantes les unes des autres (en plus, une injection de 1 cc. de solution à 20 % de salicylate de soude). Il était alors très amélioré ; la tumeur faisait seulement une petite tuméfaction, de consistance pâteuse, au milieu de laquelle on sentait, par la palpation, de petites nodosités.

Plus tard, après un an environ, la réduction était encore plus nette et les nodosités avaient disparu (Fig. 4) ; il ne reste qu'une petite infiltration, à peine reconnaissable par la palpation, et avec le bras en adduction.

III. — M. C. S., âgée de 20 jours, enregistrée à la consultation (Hôpital de Saint-Antoine) le 3 mars 1936, pour un grand lymphangiome congé-

nital. Il siège dans la région cervicale gauche, occupant un large espace, ayant pour limites : en avant, une ligne qui descendant de la région parotidienne jusqu'au menton, et de là jusqu'au manubrium sternal, suit la clavicule, contourne la région scapulaire et, longeant la nuque, remonte jusqu'au pavillon de l'oreille (Fig. 5). Cette tumeur, recouverte de peau fine, sans arborisations, est un peu lobée par deux faibles sillons verticaux ; elle contient un liquide séreux qui sort facilement par ponction.

Après la première injection de citrate (3 cc.), il semble que déjà la réduction s'opère ; elle est nette après la deuxième, faite deux semaines plus tard, et pour cela la troisième n'est que de 1 cc. La tumeur avait alors le volume d'une mandarine. La tendance à la guérison était si notable qu'on ne fit plus de traitement. Deux mois n'avaient pas passé sur la première injection que la tumeur était réduite à un petit nodule, à peine perceptible à la palpation (Fig. 6). Aujourd'hui (juillet 1938), ce sont seulement les plis de la peau qui dénoncent l'existence antérieure de la masse tumorale (Fig. 7).

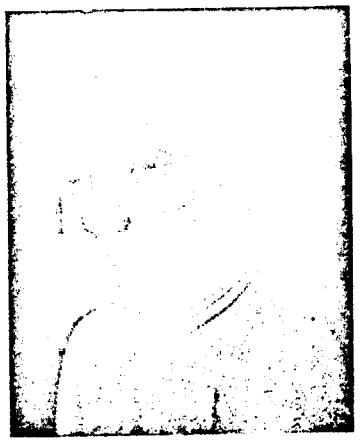

Fig. 7

Telles sont, dans leur simplicité, nos trois observations. Elles montrent que le citrate de soude, en solution saturée, convenablement injecté, réduit les lymphangiomes de telle façon qu'on peut les considérer comme guéris.

La guérison vient après un nombre variable d'injections, qui a été très petit (3 seulement) dans notre troisième observation ; chez ce malade, la régression a été si extraordinairement précoce que nous pensons être tombé sur un cas (certainement rare) avec tendance à la sclérose spontanée ; mais, dans ces conditions mêmes, l'efficacité de la méthode thérapeutique n'est pas douteuse.

La réduction du lymphangiome correspond à une vraie guérison, comme le prouve la stabilité des résultats, après quelques années.

RECUEIL DE FAITS

BRONCHITE CAPILLAIRE D'ORIGINE DIPHTÉRIQUE ET OTITE SECONDAIRE DUE AU BACILLE DE LOEFFLER

par M. le Dr J.-M. PAEZ DE LA TORRE

J'ai eu l'occasion d'observer un cas de bronchite capillaire d'origine diphtérique et, à cause de sa rareté, j'ai résolu de le publier dans les « *Archives de médecine des enfants* ». L'observation fut recueillie dans mon service de la Protection de l'Enfance à Tucumán. Voici l'historique clinique :

R. I..., âgé de trois mois, est admis dans le service le 30 avril 1938.

Antécédents familiaux : le père tuberculeux, la mère bien portante, 4 enfants vivants, 6 morts, pas de fausses couches.

Antécédents personnels : allaitement mixte sans inconvénients. Etat actuel : poids 6.400 gr., taille 60 centimètres. Léger rachitisme au thorax, pannicule adipeux conservé, turgence légèrement diminuée.

Appareils circulatoire, respiratoire et digestif normaux.

Système nerveux : hyperexcitable (diathèse névropathique).

Réaction de Mantoux : positive dans les 48 heures.

Radioscopie du thorax : ombres ganglio-pulmonaires hilaires très discrètes.

Réaction de Kahn chez la mère : négative.

Mai 11. Température : 37°4. La mère apporte l'enfant parce qu'il tousse fréquemment. Le nourrisson est agité et présente des accès de toux. Pleurs et toux conservent leur timbre normal. Il y a du tirage sus- et sous-sternal. Difficulté pour l'alimentation, selon la mère. Faciès légèrement cyanotique. L'examen des poumons nous donne des râles humides peu nombreux, disséminés dans les deux côtés et quelques sibilances. L'examen de la gorge est rendu difficile par d'abondantes sécrétions muqueuses et visqueuses qui

ON THE NOTION OF TOXICITY: THE TOXICITY OF TRISODIUM CITRATE IN INTRAVENOUS INJECTION

by MM. R. Charonnat and P. Lechat (*)

(*) Report presented before the Academy of Pharmacy, session of Oct. 25,1950.

Through its multiple uses sodium citrate can pass for one of the most inoffensive drugs and the Codex did not think of including it in the table reserved for dangerous substances. Its injection into the veins is even so common that the title of this article may seem unusual.

The most recent treatises or manuals of pharmacy or pharmacology generally entertain correct reservations regarding the innocuity of these intravenous injections and recommend that they be used slowly.

In searching for pyrogens in solutions of sodium citrate for transfusion, by the official test, we have been struck by the regularity and the violence of the accidents caused,and by the importance of this phenomenon (1)

These accidents have been observed over a long time and form the subject of already numerous studies(1)

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(1) We shall not attempt to establish an exhaustive bibliograph of the toxicity of citrates; we should have to go back to 1845 (C. G. MITSCHERLICH), well beyond the references which are given normally; we shall quote only some essential reports.

The frequency and the gravity of accidents due to too rapid injections of various drugs (general or local anaesthetics, alkaline salts, metal salts) leads us to insist on this question in the most typical case, that of sodium citrate. The toxic action of sodium chloride was known and used in laboratories since long ago; a whole series of tragedies had to take place before people became concerned; the same vigilance must be exercised for trisodium citrate.

The question of shock in injections is still not well known and calls for much simultaneous research on the part of clinicians and pharmacists. Trisodium citrate is a particularly favorable material for study; accidents are due to it;

it remains to coordinate the facts already observed, to understand them and to avoid them.

I. - CHARACTERISTICS OF THE TOXICITY OF TRISODIUM CITRATE IN INTRAVENOUS INJECTIONS.

When a sterile aqueous and apyrogenous solution of trisodium citrate is injec in the marginal vein of the ear of a rabbit, at usual concentrations and at the speed prescribed for the pyrogens tests, a succession of phenomena is quickly observed; they have been described many times, though not always concistently; one report (2) deals with temperature riese, attributable to a pyrogen; the inte pretation of accidents is fairly divergent; we note here a succession of perceptible manifestations:

a) Shaking of the head, followed by trembling of the rest of the body;

b) Cries, sometimes later;

c) Convulsions: almost always a sudden jump, the animal inverts its normal curvature, with the head curving towards the back;

d) Pedaling movements;

e) Protrusion of the eyes;

f) dyspnea, then CHEYNE-STOKES respiration;

g) Jaw open to abnormal extent, abundant salivation, relaxation of the sphincters

h) Arrested respiration, followed by heart arrest.

A part of these phenomena is identified with accidents of the cardiozolic crisis (3),but, an essential difference, they are not avoided by the use of anti epileptic agents. We shall return to the comparison with colloidoclassical shock.

If the injection has not been too rapid and the dose too strong, in phase f the animal rallies spontaneously after two to ten minutes, remains a little uncoordinated and quickly resumes a normal life.

A whole series of non-fatal identical crises can be produced in a same animal by introducing the citrated solution slowly enough and by suspending the injection in time.

Here are some numerical data extracted from numerous personal experiments. The weights and concentrations of trisodium citrate are expressed in anhydrous salt (2).

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(2) The uncertainty existing in the work, concerning the hydration of the trisodium citrate used, destroys all accuracy in the comparisons. The officinal citrate at 5.5 OH_2 is efflorescent; the one in our collection loses 11.9% water at 100° instead of 17.7; we have used the salt at 2 molecules of water, which is officinal in the United States.

The anticoagulant solution of U.S.P. XIV is at 2.3 - 2.7% of salt dihydrated with 0.85 - 0.95 of ClNa %. In France solutions are used which go from 2.5 to 4% and even more, but in hydrated salt of the Codex at 5.5 OH_2. Perhaps this explains the insufficient coagulating powers of certain solutions.

Salt at 2 OH_2	Anhydrous salt x 1.14
Salt at 5.5 OH_2	Anhydrous salt x 1.38

The isotonic solution of trisodium citrate, according to the cryoscopy performed at the laboratory of Pharmacie Centrale des Hôpitaux by our co-worker J. MAILLARD, contains 2.5% of anhydrous salt (3.46 of salt Codex 1938).

Here are the figures found:

Delta	Anhydrous salt	Salt at 2 OH_2	Salt at 5.5 OH_2
0°.50	2.19	2.50	3.03
0°.56	2.50	2.85	3.46
0°.72	3.32	3.80	4.59

HEDON, 1917 (10) has fixed the isotonia at 1.5% with respect to the red blood corpuscles of the rabbit.

It seems, from the results of the table, that a dose near 215 mg of anhydrous trisodium citrate is lethal, regardless of the speed of injection or the titer of the solution.

However much larger doses can be injected without fatal result:

A. Date of Experiment

B. Weight of rabbit in Kg.

C. Anhydrous citrate in mg/kg.

D. Percentage of solution.

E. Duration of the injection in seconds.

F. Degree of crisis reached, and duration

G. With acute edema of the lung, visible muscular trembling after death.

A	B	C	D	E	F
September 6, 1948	3.05	44	2.71	60	c.
September 6, 1949	2.25	60	2.71	60	f.
October 7, 1949	2.35	90	7.14	15	f, ended in 2 min.
October 27, 1949	2.45	116	7.14	150	c.
March 24, 1950	3.37	145	7.14	15	f, ended in 10 min.
March 30, 1950	1.60	145	7.14	15	f, ended in 15 min.
September 29, 1949	2.45	145	7.14	30	f.
November 14, 1949	2.50	145	7.14	45	f, ended in 15 min.
March 31, 1950	2.45	180	7.14	15	f, ended in 5 min.
October 5, 1950	2.70	180	7.14	30	f, ended in 10 min.
March 24, 1950	3.37	215	7.14	10	Death in 2 min.
March 30, 1950	1.66	215	7.14	15	Death in 4 min.
March 31, 1950	2.45	215	7.14	15	Death (1)
April 7, 1950	2.62	215	7.14	20	Death in 2 min.
October 18, 1950	2.05	215	2.71	30	Death in 30 sec.
October 18, 1950	2.35	215	21.43	30	Death in 1 min.
September 6,1949	2.05	264	2.71	180	Death at end of injection

237 mg/kg injected in three times (60 + 60 + 117 mg) distributed over five minutes (3 kg rabbit) brought the rabbit successively to stages a, c and f without death.

357 mg/kg (2.250 kg rabbit), distributed in five injections over two hours produced only a few convulsions; on the other hand 393 mg/kg (2.700 kg rabbit) in two injections of thirty seconds at ten minutes' interval produced death.

792 mg/kg (2.750 kg female rabbit) in discontinuous injections distributed over two hours caused only some respiratory troubles; same results with 880 mg/kg (1.700 kg rabbit) in eight separate, slow injections distributed over 1-1/2 hour.

These results illustrate the influence of the speed of injection; we shall compare these to data taken from previous reports: SALANT and WISE (4) have pointed out this influence already since 1916; (most of our experiments were carried out with shorter durations of injection, nearer to practical conditions):

70 mg/kg in eighty-five seconds suffice to trigger the crisis in the rabbit;

460 mg/kg in 160 minutes or 700 in 74 minutes in discontinuous injections cause repeated crises without fatal result;

1100 mg/kg at a speed of 35 mg per minute, with some periods of rest, or 1600 mg/kg in 3-1/2 hours, despite several stops, determine the deaths of the rabbits (3).

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(3) The quantities seem expressed in anhydrous salt, although the report does not say so for all experiments.

NORMET (1924 [5] has established three limit doses for the rabbit: less than 90 mg/kg, no accident; from 105 to 120 mg/kg, troubles with healing; more than 120 mg/kg, certain death. These results seem in serious disagreement with those of SALANDand WISE and with ours; the absence of indication on the time of injection and on the hydration of the salt does not make it possible to compare them with any other value; they let one think that the injections performed at that time were very rapid.

We have found the way to avoid crisis and death without suspending the injection; one merely adopts a fairly low concentration: the injection of a solution at 0.88% of anhydrous trisodium citrate continued without stopping during four hours at the rate of 1 mg per minute and per kg of animal (continuous perfusion apparatus), or 264 mg/kg, has caused only slight respiratory troubles, perhaps due to the volume injected.

It is therefore useless to indicate a toxic dose for the sodium citrate if one does not at the same time indicate the speed or the rhythm of the intravenous injection and the concentration of the solution. For sodium citrate and for many other toxic materials, a fatal dose of a LD_{50} (4) expressed only by a mass (even referred to the unit of body weight) is an insufficient characteristic. It is

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(4) LD_{50} is the dose which kills half the animals in a series

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worth about as much as a specific rotary power, as used to be given, without indication of solvent or of concentration.

The influence of time is negligible only for very rapid injections and very severe intoxications; the LD_{50} then tends toward a limit value; the variable values are the most interesting to be considered in practice. The LD_{50} curve in function of the duration of injection is yet unknown for citrate in the rabbit; one only knows (7) the value, 449 mg/kg in anhydrous salt for five minutes; the limit value must be near 200 mg for a very rapid injection (Fig. 1).

It is necessary to plot the graph of toxicity in function of the duration of the injection; in principle, in the example of trisodium citrate, the diagram must have the following form (Fig. II); the durations of injection are shown in abscissae, and the percentage of animals displaying the chosen effect is shown in ordinates: death or crisis; for a quantity q injected at the concentration c during a time t, 1% of crises are observed, of which m% are fatal, n% of the animals escaping the crisis; with the different values of t one determines two curves (Fig. III) defining three regions: A, crisis and death; B, non-fatal crisis; C, no crisis. The two curves are characteristic of q at concentration c.

We did say "in principle", for we are not yet in a position to give the experimental curve: one would have to sacrifice a large number of animals and especially, at the concentrations we used, many suspensions of injection proved necessary to avoid death; this discontinuity is not without influence on the values to be defined.

II.- RESEARCH ON THE CAUSES OF TOXICITY OF TRISODIUM CITRATE IN INTRAVENOUS INJECTION.

After these considerations of general toxicology, let us examine the phenomenon more closely..

For NORMET (1924 [5]), whose thesis was taken up by GOIA and PETRI in 1927 (6), trisodium citrate causes "a shock phenomenon by exciting action on the sympathetic nervous system and this action is due to the sodium ion and not to the citric radical". The hypothesis is no longer defensible in this form after the experiments of GRUBER and HALBEISEN (1948)(7). These American

authors determined the LD_{50} of citric acid and of its three mono-, di- and trisodium salts, in intravenous injection at a well specified speed, in the rabbit and in other animals. In order to facilitate comparison, the toxic doses are expressed in millimolecules per kilogram of animal.

At the intravenous injection speed of 0 millimol., 358 in 0.75 ml (solution at 17% of salt at 5.5 OH_2), per minute, toxicity with respect to the rabbit is determined as follows:

Citric acid	1.73 millimol/kg
Monosodium citrate	1.75 "
Disodium citrate	1.77 "
Trisodium citrate	1.74 (5) "

(5) Or 449 mg. of anhydrous trisodium citrate injected in five minutes.

These 4 values are practically equal; the toxicity is quite characteristic of the citric ion.

Japanese authors (8) searching for an anticoagulant in replacement of sodium citrate, which was in short supply in their country during the world conflict, found an excellent one in sodium bicarbonate; they note that the incidents upon injection are the same as those observed with the citrate; these incidents must be compared in detail in order to establish what is due to each of the ions.

For other authors the incidents caused in animal and man by intravenous injections of sodium citrate are so comparable to those of calciprivic tetanine that they must be referred to the disappearance of calcium ions from the blood. Some see there a simple precipitation, others a deionization.

Calcium citrate is little soluble in water: 960 mg per liter at 25°; in fact serum proteins prevent its precipitation. The precipitation of calcium is more complete with oxalate, fluoride and phosphate of sodium which salts, at a sufficient dose, also cause death in the rabbit.

The term deionization is improper; it is the Italian SABATTANI (9), who, in 1901, advance this notion of disappearance of calcium ions under a new form: a calcocitrate. In calcium citrate the Ca ions are already concealed, as is said

in the chemistry of complexes (the saturated solution does not have the characteristic flavor of calcium salts), but this concealment is incomplete, for the saturated solution precipitates immediately by addition of ammonium oxalate. In the serum, proteins enter into play: if one puts trisodium citrate and calcium chloride together in appropriate quantities, in water in one part and in serum in the other, than adds an excess of ammonium oxalate, the precipitation of calcium oxalate is delayed by several hours in the serum medium.

In intoxication by trisodium citrate in intravenous injection, the reversibility of the phenomenon must be stressed. If the injection is opportunely interrupted, imminent death is quickly put off and the animal seems to have no recollection of the crisis; he will react in the same manner fifteen minutes later. The arterial pressure curve well reflects this self-defense; the pressure drops very quickly and rises again just as rapidly (experiment performed on a 13 kg dog; 110 mg anhydrous citrate per kilogram injected in ten seconds).

With the hypothesis of calcium concealment, the explanation is self-evident; HEDON (1917)(10) invoked the defense of the organism by mobilization of the calcium ions from the tissues to explain that five times more trisodium citrate is needed "in vivo" than "in vitro", if one wishes to make the same mass of blood incoagulable; one should rather invoke a rapid diffusion of the active form of the calcium or else a transposition of the protein-calcocitrate complex with release of calcium cations.

III.- MEANS OF PREVENTING ACCIDENTS DUE TO TRISODIUM CITRATE.

Few means outside of dilution, slowness or fractionating of injections make it possible to prevent these accidents; we shall study pharmacodynamic means in another report[6].

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(6) CHARY and ARDRY (11) have recently prescribed the protection of rabbits, intended for research of pyrogens in trisodium citrate solutions, through the prior ingestion of calcium gluconate. In most cases the crisis is averted. The observation is interesting; yet one should make certain that the calcium gluconate has no effect on the thermic reactions of the animals; it seems much simpler to dilute the solution to be tested with distilled water, a physiolog-

ical solution or a calcium chloride solution known to be free of pyrogens (1).

The hypothesis of SABBATANI (1901) led HEDON (1917) to use calcium chloride as an antagonist of trisodium citrate; relatively little calcium is needed to bring a rabbit back from imminent death to viable survival; 570 mg (in anhydrous salt, 10% solution of officinal salt) being injected in the vein of the ear of a rabbit weighing 2.500 kg cause a violent reaction sufficient to arrest respiration. By injection of 12 ml of calcium chloride at 1%, or 60 mg of anhydrous salt, the animal recovers, breathes in a few seconds, shocks and paralysis disappear, and disintoxication is complete (HEDON).

Based on the above we have conceived a fairly curious experiment.

7 ml of officinal trisodium citrate at 10% injected in thirty seconds regularly kill a rabbit weighing approximately 2.500 kg. 7 ml of calcium chloride at 10% injected in the same manner also regularly kill a rabbit weighing 2 to 3 kg but by slow injection (150 seconds) the animal is not killed; the convulsive crisis surprisingly resembles the citric crisis attributed to hypocalcemia.

Let us mix both solutions; this is quite an incompatibility. In ten minutes, at ordinary temperature, there begins a precipitation of calcium citrate which becomes massive in one hour. We inject the liquid before any precipitation, as rapidly as possible considering the diameter of the marginal vein of the ear of the rabbit (2.300 kg)(7), or approximately thirty seconds, twice as rapidly as

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(7) If proportionality existed with respect to the weights this intravenous flow would correspond to almost 1 liter/minute for man.

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the individual solutions; no shock is observed upon inection, the rabbit remains a little immobile but almost immediately resumes normal activity; no trouble appears during the subsequent days.

The singularity of these two sudden toxic actions which cancel one another has been verified with other rabbits, for other proportions and with different calcium salts; we shall return to this antagonism in another report.

CONCLUSIONS

Several conclusions can be drawn from these experiments:

1. The toxicity of trisodium citrate in intravenous injection is essentially a function of the speed of the injection and of the concentration of the solution.

2. A weak concentration of the solution and a slow injection permit a continuous inoffensive administration in the rabbit.

3. The accidents observed are due neither to hypertonia, nor to sodium ions, nor to an overload of the right heart recently mentioned by CHARY and ARDRY (11), since we can increase this overpressure with impunity.

4. It is possible to suddenly inject, without accident, a hypertonic solution in the vein of a rabbit; this would lead us to review which concentrated solutions permit a sudden injection and which substances surely cause accidents. On the theoretical level, such a research will render great services in the study of shock during intravenous injections. On the practical level, one should not depart from a strict rule: in human clinical work intravenous injections should be given very slowly.

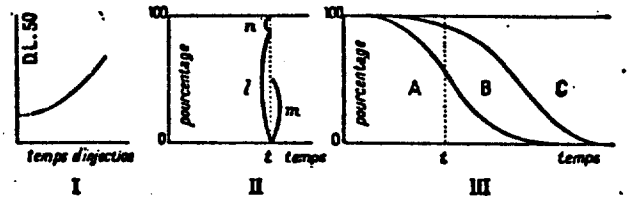

Fig 1.

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TABLEAU III. — *Vitesse d'absorption de l'ésérine introduite par voie sous-cutanée chez le Rat (0 mg.,1) par animal) en présence de doses variables d'hyaluronidase injectées 5 minutes auparavant.*

QUANTITÉ d'hyaluronidase (en V.R.U.)	NOMBRE d'animaux	TEMPS de latence (M + σ, en min.)	TEMPS d'action maximum (M + σ, en min.)
0,05	4	13,5 ± 3,8	29 ± 6,0
0,10	4	10,5 ± 3,1	35 ± 2,6
0,20	4	7,5 ± 1,7	20 ± 5,7
2,0	4	7 ± 2,2	17 ± 10,5
10,0	4	8 ± 1,8	19 ± 5,4

Nous pouvons déduire de ces expériences qu'à partir de la concentration de 0,20 V. R. U. par centimètre cube, l'hyaluronidase exerce son effet maximum sur la diffusion. L'accélération de l'absorption est telle que la valeur du temps de latence est passée de dix-huit à sept minutes (chiffres moyens), c'est-à-dire que ce temps s'est trouvé raccourci de près de 60 %. Si l'on tient compte des chiffres précédemment publiés [3], on note même que l'ésérine, administrée par voie sous-cutanée en présence d'hyaluronidase, pénètre dans l'organisme du Rat plus rapidement que par injection intra-péritonéale.

En conclusion, les résultats de nos expériences apportent quelques précisions concernant l'effet de l'hyaluronidase sur la diffusion d'une substance médicamenteuse au sein du tissu conjonctif ; elles mettent en évidence la brièveté de cette action et l'importance de la concentration des solutions d'hyaluronidase utilisées.

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Sur la notion de toxicité : la toxicité du citrate trisodique en injection intraveineuse,

par MM. R. CHARONNAT et P. LECHAT (*).

A travers ses multiples emplois, le citrate de sodium peut passer pour l'un des médicaments les plus inoffensifs et le Codex n'a pas songé à le mettre dans un tableau réservé aux substances dangereuses. Son injection dans les veines est même si courante que le titre de ce travail peut paraître insolite.

Les traités ou manuels de pharmacie ou de pharmacologie les plus récents font, en général, de justes réserves sur l'innocuité de ces injections intraveineuses et recommandent de les effectuer avec lenteur.

En recherchant les pyrogènes dans les solutions de citrate de sodium pour transfusion, par le test officiel, nous avons été frappés de la régularité et de la violence des accidents provoqués, de l'importance du phénomène [1].

Ces accidents ont été remarqués depuis longtemps et ont fait l'objet d'études déjà nombreuses (1).

La fréquence et la gravité des accidents dus à des injections trop rapides de médicaments variés (anesthésiques généraux ou locaux, sels alcalins, sels métalliques) nous amènent à insister sur cete question dans le cas le plus typique, celui du citrate de sodium. On connaissait et utilisait depuis longtemps, dans les laboratoires, l'action toxique du chlorure de sodium ; il a fallu toute une série de drames pour qu'on s'en inquiète ; la même vigilance doit être assurée pour le citrate trisodique.

La question du choc dans les injections est encore très mal connue et appelle de nombreuses recherches simultanées de la part des cliniciens et des pharmaciens. Le citrate trisodique est un matériel d'étude particulièrement favorable ; des accidents lui sont dus ; il reste à coordonner les faits déjà relevés, à les comprendre et à les éviter.

I. — CARACTÉRISTIQUES DE LA TOXICITÉ DU CITRATE TRISODIQUE EN INJECTIONS INTRAVEINEUSES.

Quand on injecte dans la veine marginale de l'oreille d'un Lapin une solution aqueuse stérile et apyrogène de citrate trisodique, aux concentrations usuelles et à la vitesse prescrite pour l'essai des pyrogènes, on observe très rapidement une suite de phénomènes ; ils ont

(*) Mémoire présenté à l'Académie de Pharmacie, séance du 25 octobre 1950.

1. Nous ne chercherons pas à établir une bibliographie exhaustive de la toxicité des citrates ; il faudrait la faire remonter jusqu'en 1845 (C. G. MITSCHERLICH), bien au delà des références données habituellement ; nous ne citerons que quelques mémoires essentiels.

été maintes fois décrits, pas toujours de façon concordante ; dans une relation [2] figure l'élévation thermique, attribuable à un pyrogène ; l'interprétation des accidents est assez divergente ; nous notons ici la succession des manifestations perceptibles :

a) Secousses de la tête, puis tremblement du reste du corps ;

b) Cris, parfois plus tardifs ;

c) Convulsions : presque toujours saut brutal, l'animal inverse sa courbure naturelle, la tête partant vers le dos ;

d) Mouvements de pédalage ;

e) Exophtalmie ;

f) Dyspnée, puis respiration de CHEYNE-STOKES ;

g) Mâchoire démesurément ouverte, salivation abondante, relâchement des sphincters ;

h) Arrêt de la respiration, suivi de l'arrêt du cœur.

Une partie de ces phénomènes s'identifie avec les accidents de la crise cardiazolique [3] mais, différence essentielle, ils ne sont pas évités par l'emploi des antiépileptiques. Nous reviendrons sur la comparaison avec le choc colloïdoclasique.

Si l'injection n'a pas été trop rapide et la dose trop forte, à la phase *f* l'animal se ranime spontanément après deux à dix minutes, garde un peu d'incoordination et reprend vite une vie normale.

On peut produire chez le même animal toute un série de crises identiques non mortelles, si l'on sait introduire assez lentement la solution citratée et suspendre à temps l'injection.

Voici quelques données numériques extraites de nombreuses expériences personnelles. Nous avons exprimé les poids et les concentrations de citrate trisodique en sel anhydre (²).

2. L'incertitude qui règne dans les travaux, sur l'hydratation du citrate trisodique employé, enlève toute précision aux comparaisons. Le citrate officinal à 5,5 OH_2 est efflorescent ; celui de notre collection perd 11,9 % d'eau à 100° au lieu de 17,7 ; nous avons utilisé le sel à 2 molécules d'eau, plus stable, officinal aux U. S. A.

La solution anticoagulante de l'U. S. P. XIV est à 2,3-2,7 % de sel dihydraté avec 0,85-0,95 de ClNa %. En France, on emploie des solutions qui vont de 2,5 à 4 % et même plus, mais en sel hydraté du Codex à 5,5 OH_2. Peut-être faut-il trouver là l'explication de l'insuffisance du pouvoir coagulant de certaines solutions.

Sel à 2 OH_2 . . . . . Sel anhydre × 1,14
Sel à 5,5 OH_2 . . . . . Sel anhydre × 1,38

Le soluté isotonique de citrate trisodique, d'après la cryoscopie effectuée au laboratoire de la Pharmacie Centrale des Hôpitaux par notre collaborateur J. MAILLARD, contient 2,5 % de sel anhydre (3,46 de sel Codex 1938).

Voici les chiffres trouvés :

Δ	SEL ANHYDRE	SEL A 2 OH_2	SEL A 5.5 OH_2
— 0°,50.	2,19	2,50	3,03
— 0°,56.	2,50	2,85	3,46
— 0°,72.	3,32	3,80	4,59

HÉDON, 1917 [10], a fixé l'isotonie à 1,5 % vis-à-vis des hématies du Lapin

Il semble, d'après les résultats du tableau, qu'une dose voisine de 215 mg. de citrate trisodique anhydre est mortelle, quels que soient la vitesse d'injection et le titre du soluté.

On peut cependant injecter des doses beaucoup plus élevées sans issue fatale :

A B C D E F

DATE de l'expérience	POIDS du lapin en kg.	CITRATE anhydre en mg./kg.	POURCENTAGE de la solution	DURÉE en secondes de l'injection	DEGRÉ de crise atteint et durée
6 septembre 1949. .	3,03	44	2,71	60	*c*.
6 septembre 1949. .	2,25	60	2,71	60	*f*.
7 octobre 1949 . .	2,35	90	7,14	15	*f*, finie en 2 min.
27 octobre 1949 . . .	2,45	116	7,14	150	*c*.
24 mars 1950	3,37	145	7,14	15	*f*, finie en 10 min.
30 mars 1950	1,60	145	7,14	15	*f*, finie en 15 min.
29 septembre 1949. .	2,45	145	7,14	30	*f*.
14 novembre 1949. .	2,50	145	7,14	45	*f*, finie en 15 min.
31 mars 1950	2,45	180	7,14	15	*f*, finie en 5 min.
5 octobre 1950 . . .	2,70	180	7,14	30	*f*, finie en 10 min.
24 mars 1950 . .	3,37	215	7,14	10	Mort en 2 min.
30 mars 1950	1,66	215	7,14	15	Mort en 4 min.
31 mars 1950 . . .	2,15	215	7,14	15	Mort (¹).
7 avril 1950 . . .	2,62	215	7,14	20	Mort en 2 min.
18 octobre 1950 . . .	2,05	215	2,71	30	Mort en 30 sec.
18 octobre 1950 . . .	2,35	215	21,43	30	Mort en 1 min.
6 septembre 1949. .	2,05	264	2,71	180	Mort à la fin de l'injection.

1. Avec œdème aigu du poumon, trémulations musculaires visibles après la mort.

237 mg./kg. injectés en trois fois (60+60+117 mg.) répartis sur cinq minutes (lapin de 3 kg.), ont amené successivement le lapin aux stades *a*, *c* et *f* sans mort.

357 mg./kg. (lapin de 2 kg.,250), répartis en cinq injections sur deux heures, n'ont produit que quelques convulsions ; par contre, 393 mg./kg. (lapin de 2 kg.,700) en deux injections de trente secondes à dix minutes d'intervalle ont provoqué la mort.

792 mg./kg. (lapine de 2 kg.,750) en injections discontinues réparties sur deux heures n'ont amené que des troubles respiratoires ; mêmes résultats avec 880 mg./kg. (lapin de 1 kg.,700) en huit injections séparées, lentes, réparties sur une heure trente.

Ces résultats illustrent l'influence de la vitesse d'injection ; nous les rapprocherons de quelques données relevées dans les mémoires antérieurs : SALANT et WISE [4] ont souligné dès 1916 cette influence ; (la plupart de nos expériences ont été faites avec des durées d'injection plus courtes, plus voisines des conditions pratiques) :

70 mg./kg. en quatre-vingt-cinq secondes suffisent à déclancher la crise chez le lapin ;

460 mg./kg. en cent soixante minutes ou 700 en soixante-quatorze minutes en injections discontinues provoquent des crises répétées sans issue fatale ;

1100 mg./kg. à la vitesse de 35 mg. par minute, avec quelques répits, ou 1600 mg./kg. en trois heures trente malgré plusieurs arrêts, déterminent la mort des lapins (3).

Normet (1924 [5]) a établi trois doses limites pour le lapin : au-dessous de 90 mg./kg. pas d'accident, de 105 à 120 mg./kg. troubles avec guérison, > 120 mg./kg., mort certaine. Ces résultats paraissent en désaccord grave avec ceux de Salant et Wise et avec les nôtres ; l'absence d'indication sur le temps d'injection et sur l'hydratation du sel ne permet de les comparer à aucune autre valeur ; ils laissent penser que les injections faites alors ont été très rapides.

Nous avons trouvé le moyen d'éviter la crise et la mort sans suspendre l'injection ; il suffit d'adopter une concentration assez faible : l'injection d'une solution à 0,88 % de citrate trisodique anhydre poursuivie sans arrêt pendant quatre heures à raison de 1 mg.,1 par minute et par kilogramme d'animal (appareil à perfusion continue), soit 264 mg./kg., n'a provoqué que de légers troubles respiratoires, peut-être dus au volume injecté ?

Il est donc vain d'indiquer une dose toxique pour le citrate de sodium si on n'indique pas en même temps la vitesse ou le rythme de l'injection intraveineuse et la concentration du soluté. Pour le citrate de sodium et bien d'autres toxiques, une dose mortelle ou une D. L. 50 (4) qui ne s'exprime que par une masse (même rapportée à l'unité de poids corporel) est une caractéristique insuffisante. Elle ne vaut guère mieux qu'un pouvoir rotatoire spécifique, comme on le donnait autrefois, sans indication de solvant, ni de concentration.

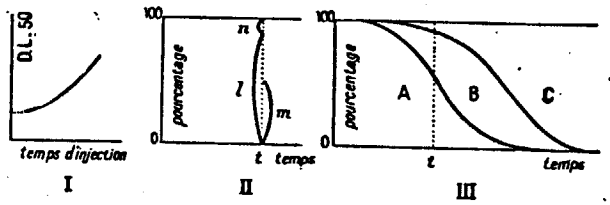
L'influence du temps n'est négligeable que pour les injections très rapides, les intoxications très sévères ; la D. L. 50 tend alors vers une valeur limite ; ce sont les valeurs variables qui sont les plus intéressantes à considérer en pratique. La courbe de la D. L. 50 en fonction de la durée d'injection est encore inconnue pour le citrate, chez le lapin ; on connaît seulement [7] la valeur, 449 mg./kg. en sel anhydre pour cinq minutes ; la valeur limite doit être voisine de 200 mg. pour une injection très rapide (fig. I).

Il faudra dans un certain nombre de cas dresser le graphique de la toxicité en fonction de la durée d'injection : en principe, dans

3. Les quantités paraissent exprimées en sel anhydre, bien que le mémoire ne le dise pas pour toutes les expériences.
4. D. L. 50 est la dose qui tue la moitié des animaux de la série.

l'exemple du citrate trisodique, le diagramme doit avoir l'allure suivante (fig. II) ; en abscisses sont portées les durées d'injection, en ordonnées le pourcentage d'animaux manifestant l'effet choisi : mort ou crise ; pour une quantité q, injectée à la concentration c, en un temps t, on observe l % de crises, dont m % de mortelles, n % d'animaux échappant à la crise ; avec les différentes valeurs de t on détermine deux courbes (fig. III) délimitant trois régions : A, crise et mort ; B, crise non mortelle ; C, pas de crise ; les deux courbes sont caractéristiques de q à la concentration c.

Nous avons dit, en principe, car nous ne sommes pas encore en mesure de donner la courbe expérimentale : il faudra sacrifier un grand nombre d'animaux et surtout, aux concentrations où nous avons opéré, plusieurs suspensions d'injection se sont montrées néces-



saires pour éviter la mort ; cette discontinuité n'est pas sans influence sur les valeurs à déterminer.

II. — Recherches sur les causes de la toxicité du citrate trisodique en injection intraveineuse.

Après ces considérations de toxicologie générale, examinons de plus près le phénomène.

Pour Normet (1924 [5]), dont la thèse a été reprise par Goia et Petri en 1927 [6], le citrate trisodique provoque « un phénomène de choc par action excitante sur le système nerveux sympathique et cette action est le fait de l'ion sodium et non du radical citrique ». L'hypothèse n'est plus défendable sous cette forme à la suite des expériences de Gruber et Halbeisen (1948) [7]. Ces auteurs américains ont déterminé la D. L. 50 de l'acide citrique et de ses trois sels mono, di- et trisodique, en injection intraveineuse à une vitesse bien précisée, chez le lapin et d'autres animaux ; pour faciliter la comparaison, les doses toxiques sont exprimées en millimolécules par kilogramme d'animal.

A la vitesse d'injection intraveineuse de 0 millimol.,358 en 0,75 ml. (solution à 17 % de sel à 5,5 OH_2), par minute, la toxicité vis-à-vis du lapin s'établit ainsi :

Acide citrique	1,73	millimol /kg.
Citrate monosodique	1,76	—
Citrate disodique	1,77	—
Citrate trisodique	1,74 (5)	—

4 valeurs pratiquement égales ; la toxicité est bien caractéristique de l'ion citrique.

Des auteurs japonais [8] à la recherche d'un anticoagulant succédané du citrate de sodium, qui fit défaut dans leur pays pendant le conflit mondial, en ont trouvé un excellent dans le bicarbonate de sodium ; ils notent que les incidents à l'injection sont les mêmes que ceux observés avec le citrate ; il faudra, dans le détail, comparer ces incidents pour établir ce qui revient à chacun des ions.

Pour d'autres auteurs, les incidents provoqués chez l'animal et chez l'homme, par les injections intraveineuses de citrate de sodium, sont tellement comparables à ceux de la tétanie calciprive qu'il faut les rapporter à la disparition des ions calcium du sang ; les uns y voient une simple précipitation, les autres une désionisation.

Le citrate de calcium est peu soluble dans l'eau : 960 mg. par litre à 25° ; les protéines sériques empêchent d'ailleurs sa précipitation. La précipitation du calcium est plus complète avec l'oxalate, le fluorure et le phosphate de sodium, sels qui, à dose suffisante, provoquent aussi la mort du lapin.

Le terme de désionisation est impropre ; c'est l'Italien Sabattani [9] qui, en 1901, a dégagé cette notion de disparition des ions calcium sous une forme nouvelle : un calcocitrate. Dans le citrate de calcium les ions Ca sont déjà dissimulés, comme on dit en chimie des complexes (la solution saturée n'a pas la saveur caractéristique des sels de calcium), mais cette dissimulation est incomplète, car la solution saturée précipite immédiatement par addition d'oxalate d'ammonium. Dans le sérum, les protéines interviennent : si on met ensemble citrate trisodique et chlorure de calcium en quantités appropriées, dans de l'eau d'une part et du sérum de l'autre, puis ajoute de l'oxalate d'ammonium en excès, la précipitation de l'oxalate de calcium est retardée de plusieurs heures dans le milieu sérique.

Dans l'intoxication par le citrate trisodique en injection intraveineuse, il faut souligner la réversibilité du phénomène. Si on interrompt opportunément l'injection, la mort proche s'éloigne vite et l'animal paraît ne garder aucun souvenir de la crise : il réagira de la même façon quinze minutes plus tard. La courbe de la pression artérielle traduit bien cette auto-défense ; la pression descend très vite et remonte aussi vite (expérience faite sur un chien de 13 kg. ; 110 mg. citrate anhydre par kilogramme injectés en dix secondes).

5. Soit 449 mg. de citrate trisodique anhydre, injectés en cinq minutes.

Avec l'hypothèse d'une dissimulation du calcium, l'explication va de soi ; Hénon (1917) [10] invoquait la défense de l'organisme par mobilisation des ions calcium des tissus pour expliquer qu'il faut cinq fois plus de citrate trisodique *in vivo* qu'*in vitro*, si l'on veut rendre la même masse de sang incoagulable ; il faut plutôt invoquer une diffusion rapide de la forme active du calcium ou bien une transposition du complexe protéine-calcocitrate avec libération de cations calciques.

III. — Moyens d'empêcher les accidents dus au citrate trisodique.

Peu de moyens en dehors de la dilution, de la lenteur ou du fractionnement des injections permettent de prévenir ces accidents ; nous étudierons dans une autre note les moyens pharmacodynamiques (6).

L'hypothèse de Sabbatani (1901) a conduit Hénon (1917) à utiliser le chlorure de calcium comme antagoniste du citrate trisodique ; il faut relativement peu de calcium pour ramener un lapin de la mort imminente à la survie durable ; 570 mg. (en sel anhydre, solution à 10 % de sel officinal) étant injectés dans la veine de l'oreille d'un lapin de 2 kg.,500 provoquent une violente tétanie allant jusqu'à l'arrêt respiratoire ; par l'injection de 12 ml. de chlorure de calcium à 1 %, soit 60 mg. de sel anhydre, l'animal revient à lui, respire en quelques secondes, les secousses et la paralysie disparaissent, la désintoxication est complète (Hénon).

Nous avons là-dessus imaginé une expérience assez curieuse.

7 ml. de citrate trisodique officinal à 10 % injectés en trente secondes, tuent régulièrement un lapin de 2 kg.,500 environ. 7 ml. de chlorure de calcium à 10 % injectés de la même façon, tuent aussi régulièrement un lapin de 2 à 3 kg., mais par injection lente (cent cinquante secondes), l'animal n'est pas tué ; la crise convulsive ressemble étonnamment à la crise citrique attribuée à l'hypocalcémie.

Mêlons les deux solutions ; c'est une belle incompatibilité : en dix minutes, à la température ordinaire, commence une précipitation de citrate de calcium qui devient massive en une heure. Injectons le liquide avant toute précipitation, aussi rapidement que le permet le diamètre de la veine marginale de l'oreille du lapin (2 kg.,300) (7), soit environ trente secondes, deux fois plus vite que

6. Chany et Andry [11] viennent de préconiser la protection des lapins, destinés aux recherches de pyrogènes dans les solutés de citrate trisodique, par l'ingestion préalable de gluconate de calcium ; dans la plupart des cas, la crise est évitée ; l'observation est intéressante ; encore faudrait-il être sûr que le gluconate de calcium n'a pas d'effet sur les réactions thermiques des animaux ; il paraît beaucoup plus simple de diluer le soluté à essayer avec de l'eau distillée, un soluté physiologique ou un soluté de chlorure de calcium reconnus apyrogènes [1].

7. S'il y avait proportionnalité, quant aux poids, ce débit intraveineux correspondrait, pour l'homme, presque à 1 litre/minute.

les solutions isolées ; aucun choc n'est observé à l'injection, le lapin reste un peu immobile, mais reprend presque aussitôt une activité normale ; aucun trouble n'apparaît les jours suivants.

La singularité de ces deux actions toxiques brutales qui s'annulent a été vérifiée sur d'autres lapins, pour d'autres proportions et avec d'autres sels de calcium ; nous reviendrons sur cet antagonisme dans une autre note.

Conclusions.

Nous pouvons tirer de ces expériences plusieurs conclusions :

1° La toxicité du citrate trisodique en injection intraveineuse est essentiellement fonction de la vitesse d'injection et de la concentration du soluté.

2° Une concentration faible du soluté et une injection lente permettent une administration continue inoffensive chez le lapin.

3° Les accidents observés ne sont dus ni à l'hypertonie, ni aux ions sodium, ni à la surcharge du cœur droit invoquée récemment par Chary et Ardry [11], puisque nous pouvons augmenter impunément cette surpression.

4° Il est possible d'injecter brutalement, sans accident, une solution hypertonique dans la veine d'un lapin ; ceci nous incite à revoir quelles solutions concentrées permettent une injection brutale et quelles substances provoquent sûrement des accidents. Sur le plan théorique, une telle recherche rendra de grands services pour l'étude du choc au cours des injections intraveineuses ; sur le plan pratique, il ne faut pas se départir d'une consigne stricte : en clinique humaine les injections intraveineuses doivent être poussées très lentement.

(*Pharmacie Centrale des Hôpitaux et Hospices civils de Paris.*)

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Hémikrèse et protorrhée de l'arsenic

(*Suite des mesures*),

par MM. Ch. Lapp et J. Spieser (*).

Les recherches de M. Duranton [1], sur l'élimination urinaire de l'arsenic, ont montré qu'à de légères fluctuations près, la loi est celle d'une exponentielle décroissante en fonction du temps : la logarithmique est rectiligne sur quarante heures.

Nous en avons déduit les valeurs de l'hémikrèse et de la protorrhée pour une seule dose thérapeutique d'arséniate de sodium, d'arsénite de potassium, de cacodylate de sodium (une seule fonction exponentielle) et d'arrhénal (deux fonctions exponentielles).

A ma demande, M. Spieser [2] a étudié sur lui-même l'élimination de l'arsenic pris aux doses infra- et supra-thérapeutiques, — sans atteindre bien entendu des doses dangereuses, — pour rechercher 1° s'il existe un seuil d'élimination de l'arsenic, 2° quelle est la quantité totale de médicament éliminée, 3° quelles sont les variations de l'hémikrèse et de la protorrhée suivant la dose ingérée.

Nous avons étudié le comportement de l'arsenic sous la forme arséniate de sodium, très ionisable et très diffusible aux doses suivantes :

200, 500, 1.000, 4.000 γ d'arsenic

et sous la forme d'arrhénal, semi-complexée à :

200, 1.000, 4.000, 15.000 γ d'arsenic.

L'élimination urinaire a été suivie toutes les heures pendant les six premières heures ; toutes les deux heures pendant les huit heures suivantes, puis toutes les trois ou quatre heures jusqu'à :

40 heures pour 200 et 500 γ. 75 heures pour 4.000 γ. 88 heures pour 1.000 γ.	Arséniate de sodium.
40 heures pour 200 γ. 55 heures pour 1.000 et 4.000 γ. 80 heures pour 15.000 γ.	Arrhénal.

Chaque miction est recueillie totalement. L'arsenic y est dosé par la micro-méthode de Cribier adaptée par Duranton à l'arsenic urinaire. On mesure le volume total émis, on a donc à chaque moment la quantité totale éliminée en ajoutant les mesures successives.

Les courbes d'élimination des figures 1 et 2 tendent peu à peu vers une limite poursuivie expérimentalement jusqu'à des valeurs

(*) Mémoire présenté à l'Académie de Pharmacie, séance du 8 novembre 1950.

Source: Annales pharm. franc., June 1951, vol. IX, No. 6, p.385-9

Experimental Research on the Phenomena of Shock Observed During Intravenous Injections

I. Cases of Sodium Salts

by R. Charonnat and P. Lechat (*)

Trisodium citrate has proved an excellent means for studying the phenomena of shock induced by salt solutions in intravenous injections. In a previous communication (1), we specified the conditions of critical shock and disclosed the possibility of giving, without accident, extremely drastic intravenous injections by combining two injections - separately lethal for the rabbit - of trisodium citrate and calcium chloride.

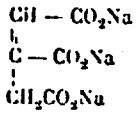
In the light of this fact, it is well to distinguish the substances which can cause shock and those whose use, at hypertonic concentration and in rapid infusion, is inoffensive, at least in relation to these shocks. Needless to say, we discard injection and consider only the accidents caused by it within minutes, or within the next hour. To clarify the toxic phenomena, the stages much be distinguished as much as possible; we shall study the first; the later vascular, hematologic, renal, hepatic complications may depend on a variety of factors other than the circumstances of the injection: Inadequate dose, individual sensitivity, pathologic contraindication, etc. The shocks envisaged are sometimes induced for therapeutic purposes. In reports which will be dealing with the analysis of shocks, we shall give a critique of earlier publications.

In this paper we shall examine two groups of sodium salts injected in the marginal vein of the ear of rabbits.

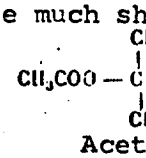
1. Salts of acids related to citric acid.

Our first research series was devoted firstly to three acids derived from citric acid; secondly to acids having, like citric acid, acid and alcohol

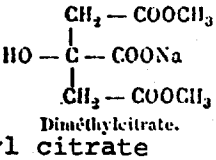
functions. We tried to determine which of the chemical characteristics of trisodium citrate intervenes in the shock without being concerned about the mechanism thereof; in this series, which is the oldest, we adopted relatively slow injections; later we chose much shorter injection times.



Aconitate



Acetyl citrate

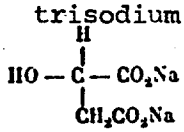


Dimethyl citrate

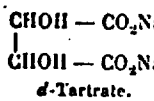
Trisodium aconitate, trisodium acetyl citrate, and monosodium dimethyl citrate, prepared in our laboratory from citric acid, were employed in very concentrated solutions, molecularly equivalent to a 30% solution of official trisodium citrate (0.844 M); 10 ml/kg, or 8.44 millimol/kg, injected in two minutes, were perfectly inoffensive; no convulsions, hardly any respiratory signs for the acetyl citrate, of which we thus used 2.52 g/kg; For this salt it is necessary to operate with a fresh solution prepared with a well controlled acetylcitric anhydride, to avoid hydrolysis and the release of a more active salt. On the other hand, 4 ml/kg of aconitate, injected in twenty-five seconds, caused a short crisis. Here 810 mg/kg of aconitate have little effect even under conditions where 200 mg/kg of citrate are already lethal.

The first two of these salts maintained the three acid functions of citric acid; the first lost only the tertiary alcohol function; the latter remains, but blocked, in the second; in the third, this alcohol function and one of the three acid functions are free. Under the adopted experimental conditions, the citric shock results from the going into play of the alcohol function and of the acid functions of the citric molecule; suppression of the alcohol function alone already attenuates the effect of this molecule considerably.

Sodium malate represents an important portion of the structure of trisodium citrate:



Malate



d-Tartrate

Mucate

In 0.106 M solution (corresponding to 3.8% of official trisodium citrate), 10 ml/kg in 165 seconds, malate causes no crisis; under the same conditions (concentration, rate of injection), the equivalent quantity of citrate is sure to be lethal.

The little soluble sodium mucate, injected only at the concentration of 3% (0.12 M) at the rate of 7.7 ml/kg in 180 seconds, produced nothing.

Sodium quinate, which has in common with citrate a group HO-C $<^{-CO_2Na}_{}$, and sodium gluconate remained without effect.

Sodium tartrate in 0.432 M solution (8.4% anhydrous salt), injected at the rate of 2.4 ml/kg in twenty seconds or 8 ml/kg in seventy-five seconds, caused no disorder. This is quite strange if one assumes with various authors that the action resides in a subtraction or precipitation of the calcium ions (2); disodium d-tartrate precipitates calcium from a dilute solution much faster and much more completely than trisodium citrate.

The hypothesis of a de-ionization of the calcium by temporary formation of a calcocitrate (3) finds support, on the other hand, in the following experiments: In a series of test tubes one places 0.5 ml of calcium chloride at 10% of officinal salt, then 10 ml of water or of sodium salt taken at the concentration 0.844 M (or 30% officinal salt for the citrate), and lastly 2 ml of ammonium oxalate 2%; the precipitation is immediate and massive in the absence of sodium salt or with dimethyl citrate, barely delayed with aconitate, substantially delayed with acetyl citrate, nothing during the first four hours with citrate. This is confirmed when using lower concentrations of the various salts studied. The formation of the calcium complex, and the temporary dissimulation of the calcium ions relative to the oxalate, so clear with citrate, is zero or negligible with dimethyl citrate, acetyl citrate, and aconitate.

2. Various sodium salts

In a second, more recent series of experiments, we gave up using the intervention of calcium in shock as a working hypothesis and injected the

sodium salts of a variety of acids, at high concentration, in more rapid injection than in the experiments of the first series. The recapitulative table gives the quantities injected per kilogram of weight of animal, expressed in milligrams of anhydrous substance and, for easier comparison, in millimol-g.; three reference experiments are indicated for trisodium citrate; one gives the minimum conditions for obtaining with this produce an incipient crisis limited to a single sign, shaking of the head; the necessary quantity is substantially lower than the values given by other authors (1) due to the faster injection.

This table calls for various comments.

Salt	Milli-mol/kg	Anhydrous salt mg/kg	% anhydrous salt in the solution	Injection time sec	Immediate result
Citrate	0.1	26	7.1	2	Start of crisis
-	0.58	150	7.1	30	Always crisis, sometimes death
-	2.3	600	21.4	30	Rapid death
Chloride	2	117	5.8	20	No crisis
-	6	351	17.5	30	" "
-	6	351	17.5	15	Start of crisis
-	6	351	17.5	5	Sudden jump & rapid death
Bromide	2	206	10	20	Accel.respir.
Iodide	2	300	15	15	No crisis
Fluoride	1	42	1.05	30	" "
Thiocyanate	1	82	8.2	15	" "
-	2	164	8.2	10	" "
Sulfate	4	568	14.2	30	" "
-	4	568	14.2	15	Crisis
Disodium phosphate	0.4	60	2	30	No crisis
- -	1.03	145	4	30	Agitation, cries, paralysis of hind section

Oxalate	0.41	56	1.87	30	No crisis
-	0.82	110	3.74	45	Strong crisis, death in 3 or 4 minutes
Tartrate	1	200	8.4	20	No crisis
-	1.5	300	8.4	30	" "
Acetate	4	328	16.4	10	Crisis of spec. kind 1)
-	6	492	16.4	20	" " "
Benzoate	2	288	14.4	20	No crisis
Phthalate	2	420	21	20	" "
Salicylate	2	320	16	20	" " ,but cries
Gentisate	2	352	17.6	20	" " , long cries
p-aminobenzoate	2	318	10	30	No crisis
p-aminosalicylate	2	348	17.4	30	" "
Camphosulfonate	2	500	10	30	" "

1) The crisis brought about by acetate is clearly different from the citric crisis; the released rabbit flops down, the four feet spread out and stiff; the respiration increases in amplitude; in one minute everything returns to normal. With fluoride, under the above codditions, a slight motor difficulty is noted already. Intoxicating with chloride, which comes closer to the citric crisis, also presents some particularities.

This must not be regarded as a final classification of the sodium salts into inoffensive salts and dangerous salts. This "in vivo physico-chemistry" is not transposable from the rabbit to human therapeutics without further systematic studies. At most one can say that with certain salts there is much less probability of accidents than with others. If one changes the conditions of use (high concentration, rapid injection), a harmless salt like sodium chloride becomes a violent poison. The rabbit is much less sensitive to its effect than the dog. In the course of research on the choleretics, one of us was able to obtain the necessary exitus of numerous dogs

with quantities of sodium chloride quite a bit lower, in due proportion, than those which kill the rabbit.

The salts which cause more or less grave accidents upon intravenous injection are not those anticipated by the consensus of all. The lethal chloride at a concentration where gentisate gives no shock at all, the toxic acetate at a dose where para-aminobenzoate is well tolerated, are rather unexpected facts.

The different pH values of the solutes do not explain these divergencies; several salts, and notably gentisate, have proved painful at a pH very close to 7, which is that of the sodium chloride solutes (this intolerance so typical of some hypertonic solutes merits a special study); trisodium citrate is slightly alkaline, but an acid citrate is no less toxic (1).

Benzoate and the five salts which follow it in the table are highly hydrotropic. At high concentration they increase the dispersion of the protein micelles and so counteract the chloride and the sulfate which tend toward flocculation; this is in agreement with one of the theories of shock, but one must guard against too general an explanation for phenomena which are visibly disparate.

Conclusions

1. Numerous sodium salts are inoffensive in the rabbit in sudden intravenous injection, at least when only the primary accidents are considered.

2. The sodium ion does not participate in the acute toxic accidents, since trisodium aconitate and trisodium acetyl citrate do not cause shock under conditions where trisodium citrate is surely lethal.

3. The hypertonicity of a solution injected into the veins is not by itself a cause of accident or pain. (Injection of a 66% glucose solution at the maximum rate compatible with the diameter of the vein and the viscosity of the solution causes no disorder in the rabbit).

4. The vascular or cardiac burden caused by a rapid massive injection is not by itself the cause of accidents.

5. The appearance of accidents, at a rather high concentration and a rather fast rate of injection, is the result of certain anions.

(Central Pharmacy of the Civil Hospices and Hospitals of Paris.)

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(*) Report submitted to the Academy of Pharmacy, session of November 8, 1950.

(Translated by Carl Demrick Associates,Inc/LH/t)

EVADOL — Analgésique central de synthèse, sans opiacé, calmant de la douleur — Ampoules de 2 cc. Suppositoires

OCYTO-NARGÉNOL — Accouchements à douleurs atténuées — Ampoules de 2 cc.

NARGÉNOL
SUPPO-NARGÉNOL — Pré-anesthésie et analgésie — Ampoules de 2 cc. Suppositoires

CINNOZYL
SUPPO-CINNOZYL — Médication tonique et reconstituante. Agent de défense contre l'infection — Ampoules de 5 cc. Suppositoires

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SCELGUT, SLOGUT, SERTY-CATGUT

LIGATURES
ANESTHÉSIQUES
AMPOULES
ACCESSOIRES

LABORATOIRES FANDRE
R. BREARD, Docteur en Pharmacie, NANCY

Tome IX. N° 6. JUIN 1951

MÉMOIRES ORIGINAUX

Recherches expérimentales sur les phénomènes de choc observés au cours d'injections intraveineuses.
I. — *Cas des sels sodiques,*

par MM. R. Charonnat et P. Lechat (*).

Le citrate trisodique s'est révélé un excellent moyen pour étudier les phénomènes de choc provoqués par les solutions salines en injections intraveineuses. Au cours d'une communication antérieure [1], nous avons précisé les conditions du choc citrique et découvert la possibilité de réaliser sans accident des injections intraveineuses extrêmement brutales en associant deux injections, séparément mortelles pour le lapin, de citrate trisodique et de chlorure de calcium.

A la lumière de ce fait, il convient de discerner les substances qui peuvent provoquer des chocs et celles dont l'emploi, à concentration hypertonique et en infusion rapide, est inoffensif, au moins vis-à-vis de ces chocs. Il va de soi que nous écartons de nos recherches les accidents lointains ; nous nous plaçons dans le cas d'une injection malencontreuse et nous n'envisageons que les accidents provoqués par elle, dans les minutes qui la suivent, au besoin dans l'heure consécutive. Pour élucider les phénomènes toxiques, il faut en distinguer autant que possible les étapes ; nous étudions la première ; les complications tardives, vasculaires, hématologiques, rénales, hépatiques, peuvent dépendre de facteurs plus variés que les circonstances de l'injection : dose inadéquate, sensibilité individuelle, contre-indication pathologique, etc. Les chocs envisagés sont quelquefois provoqués dans un but thérapeutique. Dans des mémoires qui seront consacrés à l'analyse des chocs, nous donnerons un exposé critique des travaux antérieurs.

⁂

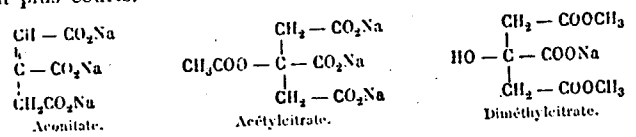
Dans ce travail nous examinerons deux groupes de sels de sodium injectés dans la veine marginale de l'oreille de lapins.

1° Sels d'acides apparentés a l'acide citrique.

Notre première série de recherches a été consacrée : 1° à trois acides dérivés de l'acide citrique ; 2° à des acides ayant comme lui

(*) Mémoire présenté à l'Académie de Pharmacie, séance du 8 novembre 1950.

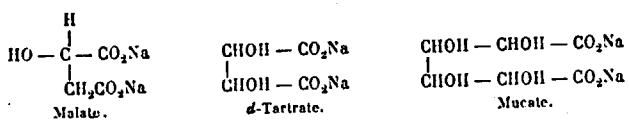
fonctions acide et alcool. Nous avons essayé de déterminer laquelle des caractéristiques chimiques du citrate trisodique intervient dans le choc sans nous préoccuper du mécanisme de celui-ci ; dans cette série, qui est la plus ancienne, nous avons adopté des injections relativement lentes ; nous avons par la suite choisi des temps d'injection bien plus courts.



L'aconitate trisodique, l'acétylcitrate trisodique et le diméthylcitrate monosodique, préparés à notre laboratoire en partant de l'acide citrique, ont été employés en solutions très concentrées, moléculairement équivalentes avec une solution à 30 % de citrate trisodique officinal (0,844 M) ; 10 ml./kg., soit 8,44 millimol./kg., injectés en deux minutes ont été parfaitement inoffensifs ; pas de convulsions, à peine quelques signes respiratoires pour l'acétylcitrate dont on employait ainsi 2,52 g./kg. ; pour ce sel il est nécessaire d'opérer avec une solution récente préparée avec un anhydride acétylcitrique bien contrôlé, pour éviter l'hydrolyse et la libération d'un sel plus actif. 4 ml./kg. d'aconitate injectés en vingt-cinq secondes, par contre, ont déterminé une courte crise : ici 810 mg./kg. d'aconitate ont peu d'effet dans les conditions mêmes où 200 mg./kg. de citrate sont déjà mortels.

Les deux premiers de ces sels ont gardé les trois fonctions acides de l'acide citrique ; le premier n'a perdu que la fonction alcool tertiaire ; celle-ci demeure, mais bloquée, dans le second ; dans le troisième, cette fonction alcool et une seule des trois fonctions acides sont libres. Dans les conditions expérimentales adoptées, le choc citrique résulte de la mise en jeu de la fonction alcool et des fonctions acides de la molécule citrique ; la suppression de la fonction alcool seule atténue déjà considérablement l'effet de cette molécule.

Le malate de sodium représente une partie importante de l'édifice du citrate trisodique :



en solution 0,106 M (correspondant à 3,8 % de citrate trisodique officinal) 10 ml./kg. en cent soixante-cinq secondes, le malate ne provoque pas de crise ; dans les mêmes conditions (concentration, vitesse d'injection), la quantité équivalente de citrate est sûrement mortelle.

Le mucate de sodium, peu soluble, injecté seulement à la concentration de 3 % (0,12 M) et à raison de 7,7 ml./kg. en cent quatre-vingts secondes n'a rien produit.

Le quinate de sodium, qui a en commun avec le citrate un groupement $HO-C<^{}_{}CO_2Na$, le gluconate de sodium sont restés sans effet.

Le tartrate de sodium en solution 0,432 M (8,4 % de sel anhydre) injecté à raison de 2,4 ml./kg. en vingt secondes ou de 8 ml./kg. en soixante-quinze secondes, n'a produit aucun trouble. Ceci est bien singulier si on admet avec divers auteurs que l'action réside dans une soustraction ou une précipitation des ions calciques [2] ; le *d*-tartrate disodique précipite le calcium d'une solution diluée, beaucoup plus vite et beaucoup plus complètement que le citrate trisodique.

L'hypothèse d'une désionisation du calcium par formation passagère d'un calcocitrate [3] trouve par contre un appui dans les expériences suivantes : dans une série de tubes à essais on place 0 ml.,5 de chlorure de calcium à 10 % de sel officinal, puis 10 ml. d'eau ou de sel sodique pris sous la concentration 0,844 M (soit 30 % de sel officinal pour le citrate), enfin 2 ml. d'oxalate d'ammonium à 2 % ; la précipitation est immédiate et massive en l'absence de sel sodique ou avec le diméthylcitrate, à peine retardée avec l'aconitate, sensiblement retardée avec l'acétylcitrate, nulle dans les quatre premières heures avec le citrate ; ceci est confirmé en employant des concentrations moindres des divers sels étudiés ; la formation du complexe calcique, la dissimulation temporaire des ions calciques vis-à-vis de l'oxalate, si nette avec le citrate est nulle ou négligeable avec le diméthylcitrate, l'acétylcitrate et l'aconitate.

2° Sels sodiques divers.

Dans une seconde série d'expériences, plus récentes, nous avons renoncé à utiliser l'intervention du calcium dans le choc comme hypothèse de travail et nous avons injecté les sels sodiques des acides les plus variés, à concentration élevée, en injection plus rapide que dans les expériences de la première série. Le tableau récapitulatif donne les quantités injectées par kilogramme de poids d'animal, exprimées en milligrammes de substance anhydre et, pour faciliter les comparaisons, en millimol.-g. ; trois expériences repères sont indiquées pour le citrate trisodique ; l'une apporte les conditions minima pour obtenir avec ce produit un début de crise limité à un seul signe, l'agitation de la tête ; la quantité nécessaire est sensi-

blement inférieure aux valeurs données par d'autres auteurs [1] en raison de l'injection plus rapide.

Ce tableau appelle divers commentaires.

SEL	MILLIMOL./KG.	SEL ANHYDRE en mg./kg.	POURCENTAGE de la solution en sel anhydre	DURÉE de l'injection en secondes	RÉSULTAT IMMÉDIAT
Citrate	0,1	26	7,1	2	Début de crise.
—	0,58	150	7,1	30	Toujours crise, parfois mort.
—	2,3	600	21,4	30	Mort rapide.
Chlorure	2	117	5,8	20	Pas de crise.
—	6	351	17,5	30	Pas de crise.
—	6	351	17,5	15	Début de crise.
—	6	351	17,5	5	Saut brusque et mort rapide.
Bromure	2	206	10	20	Accél. respir.
Iodure	2	300	15	15	Pas de crise.
Fluorure	1	42	1.05	30	Pas de crise.
Thiocyanate	1	82	8,2	15	Pas de crise.
—	2	164	8,2	10	Pas de crise.
Sulfate	4	568	14,2	30	Pas de crise.
—	4	568	14,2	15	Crise.
Phosphate disodique	0,4	60	2	30	Pas de crise.
— —	1,03	145	4	30	Agitation, cris, paralysie du train postérieur.
Oxalate	0,41	56	1,87	30	Pas de crise.
—	0,82	110	3,74	45	Forte crise, mort en 3 ou 4 minutes.
Tartrate	1	200	8,4	20	Pas de crise.
—	1,5	300	8,4	30	Pas de crise.
Acétate	4	328	16,4	10	Crise d'un type particulier (1).
—	6	492	16,4	20	Crise d'un type particulier (1).
Benzoate	2	288	14,4	20	Pas de crise.
Phtalate	2	420	21	20	Pas de crise.
Salicylate	2	320	16	20	Pas de crise, mais cris.
Gentisate	2	352	17,6	20	Pas de crise, cris prolongés.
p-aminobenzoate	2	318	10	30	Pas de crise.
p-aminosalicylate	2	348	17,4	30	Pas de crise.
Camphosulfonate	2	500	10	30	Pas de crise.

1. La crise déclenchée par l'acétate est nettement différente de la crise citrique : le lapin libéré s'affaisse, les quatre pattes écartées et raidies : la respiration augmente d'amplitude : en une minute tout redevient normal. Avec le fluorure dans les conditions ci-dessus on note déjà une légère difficulté motrice. L'intoxication par le chlorure, plus voisine de la crise citrique, présente aussi quelques particularités.

Il ne faut pas y voir une classification définitive des sels sodiques en sels inoffensifs et en sels dangereux ; cette « physicochimie *in vivo* » n'est point transposable du lapin à la thérapeutique humaine sans de nouvelles études systématiques ; on peut tout au plus dire qu'avec certains sels la probabilité d'accidents est beaucoup moins grande qu'avec d'autres ; si l'on modifie les conditions d'emploi (concentration élevée, injection rapide), un sel anodin comme le chlorure de sodium devient un poison violent ; le lapin est beaucoup

moins sensible à son effet que le chien ; l'un de nous, au cours de recherches sur les cholérétiques, a pu obtenir l'exitus nécessaire de nombreux chiens avec des quantités de chlorure de sodium bien inférieures, toutes proportions gardées, à celles qui tuent le lapin.

Les sels qui provoquent des accidents plus ou moins graves à l'injection intraveineuse ne sont pas ceux prévus par le *consensus omnium*. Le chlorure mortel à une concentration où le gentisate ne donne point de choc, l'acétate toxique à une dose où le paraminobenzoate est bien toléré sont des faits assez inattendus.

Les *p*H différents des solutés n'expliquent point ces divergences ; plusieurs sels et notamment le gentisate se sont montrés douloureux à un *p*H très voisin de 7, qui est celui des solutés de chlorure de sodium (cette intolérance si typique de certains solutés hypertoniques mérite une étude particulière) ; le citrate trisodique est légèrement alcalin, mais un citrate acide n'est pas moins toxique [1].

Le benzoate et les cinq sels qui le suivent dans le tableau sont fortement hydrotropes : à forte concentration ils augmentent la dispersion des micelles protéiques et s'opposent ainsi au chlorure et au sulfate qui tendent vers la floculation ; ceci est en accord avec l'une des théories des chocs, mais il faut se garder d'une explication trop générale pour des phénomènes qui sont visiblement disparates.

Conclusions.

1° De nombreux sels de sodium sont inoffensifs chez le lapin en injection intraveineuse brutale, au moins lorsqu'on n'envisage que les accidents primaires.

2° L'ion sodium ne participe pas aux accidents toxiques aigus, puisque l'aconitate et l'acétylcitrate trisodiques ne provoquent pas de choc dans des conditions où le citrate trisodique est sûrement mortel.

3° L'hypertonicité d'une solution injectée dans les veines n'est pas à elle seule une cause d'accident ou de douleur. (L'injection d'une solution de glucose à 66 % à la vitesse maximum compatible avec le diamètre de la veine et la viscosité de la solution ne provoque aucun trouble chez le lapin).

4° La surcharge vasculaire ou cardiaque provoquée par une injection massive rapide n'est pas à elle seule la cause des accidents.

5° L'apparition d'accidents, pour une concentration assez élevée et une vitesse d'injection assez grande, est le fait de certains anions.

(*Pharmacie centrale des Hôpitaux et Hospices civils de Paris.*)

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LA SEMANA MEDICA (Medical Weekly)

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ORIGINAL ARTICLES

ARTERIAL HYPERTENSION OBTAINED BY CISTERNAL INJECTIONS OF SODIUM NITRATE

By Doctor V. H. CICARDO
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of Medicine of Buenos Aires
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Already known is the exciter action on nervous centers produced by the injections of isotonic solutions of potassium chloride in the cephalorachidial liquid. These phenomena of excitation coincide with the marked increase of arterial pressure and respiratory changes, according to the studies carried out by -illegible- and Chvoles (1933), Vleeschowver (1935), Hermann, Jourdan, Morin and Vial (1938), Walker, Smolik and Gilson (1945), - illegible- and Wright (1947), Cicardo (1949), etc.

Similar phenomena to those produced by the injections of potassium determine the decrease of ionic calcium in the nervous centers, obtained either by injections of sodium citrate or by the perfusion of liquids without calcium, Sabbitini (1901) had proved that the application of isotonic solutions of oxalate? or citrate of sodium on the open brain of dogs determined an increase of excitability of the animal and generalized convulsions, which disappeared with the administration of calcium. These same facts were observed also in the dog, with intracisternal injections of sodium citrate by Huggins and Hasting (1933) and by Mullin, Hasting and Lees (1938), who add that beside the neuromuscular excitation, respiratory estimulation and increase of arterial pressue is produced, provoked mainly by the contracture of the somatic muscles, since they are suppressed to a large extent by means of curare. Recently, Leusen (1948) recorded during the perfusion of the cerebral ventricles

by liquids in which the concentration of potassium was increased or calcium was suppressed, arterial hypertension and reflex hyperexcitability of the basomotors. Undoubtedly, according to these experimental facts, the excitation of the nervous centers by the increase of potassium or the decrease of the ionic calcium in the cephalorachidial liquid, produces a marked increase of the arterial pressure, which in the case of sodium citrate has not been studied nor has the production mechanism been specified. Supplementing our previous work in which it was established that in the hypertension produced by the injections of potassium in the nervous centers, the sympathetic, the adrenals and the hypophysis intervened, we proceeded to study the mechanism of arterial hypertension determined by the injections of sodium citrate in the nervous centers.

METHOD

To carry out these experiments approximately 50 dogs anesthetized with Nembutal were used, whose arterial pressure was recorded at the femoral artery and respiration by means of a pneumograph in connection with a Marey capsule.

The injections of 3 per cent citrate or oxalate in the cephalorachidial liquid, preferable at the level of the magna cistern and on occasion in the lateral ventricles of the brain or at the base of the brain, in quantities which varied between 0.1 and 1 cubic centimeter.

In order to establish the mechanism of production of the arterial hypertension produced by the injection of citrate in the nervous centers, the intervention and the behavior of the sympathetic, of the adrenals and of muscular contracture were studied. In order to exclude the sympathetic medular centers experiments were carried out with animals in which the spinal medula was sentioned at the level of the 7th cervical vertebra.

FIGURE 1.- Changes in arterial pressure and in respiration by intracisternal injections of sodium citrate.

FIGURE 2.- Changes in pressure and in respiration by injections of sodium citrate in the hypothalamus.

Also studied were the characteristics of hypertension obtained by intracisternal injections of sodium citrate after blocking the sympathetic, with 1 or 2 milligrams of Ergotamine or after the injection of 30 milligrams per kilo weight of tetraethylamonium chloride which determines, according to some authors, the blocking of the ganglions of the autonomous nervous system in the same form as nicotine.

Abdominal bilateral adrenalectomy was carried out in some animals for the purpose of determining the role played by this gland in this type of nervous hypertension.

In order to discard the intervention of the neuromuscular contracture produced by the citrate, although with very small intensity in our experimental conditions, intracisternal injections were made in animals which had been fully curarized, by means of a raw extract of erythrine crista galli, maintaining them with artificial respiration. Finally, in some animals the experiments were carried out after the resection of the vagus nerves.

RESULTS

The hypertension produced by intracisternal sodium nitrate whether in the lateral ventricles or in the very thickness of the hypothalamus is marked but transitory (Figs. 1 and 2).

The rise in pressure could reach 10 centimeters of mercury and more, immediately after the injection, then maintaining itself for some minutes and dropping progressively to reach its initial value in approximately 15 minutes. In some cases the pressure maintains itself somewhat higher than at the beginning of the experiment. At the same time that the increase in pressure is recorded, generally a slight respiratory halt is recorded followed by an increase in amplitude and in respiratory frequency. If the quantity of citrate injected is somewhat high, respiratory failure and death could occur, it being possible to avoid same with artificial respiration. This respiratory failure could

come about more easily, that is in lower doses, when the injections are cisternal rather than when they are made in the other -illegible- of the cephalorachidial liquid. This fact is explained by the proximity of the respiratory center which is more easily inhibited on being reached by greater concentrations of citrate.

In the encephalic impulses are blocked (-illegible making the sentence incomplete-) excitation of the basomotory or respiratory centers are produced with less intensity and the -illegible- are each time less and end by not being produced due to the intoxication of said centers. Besides, the repetition of a dose ends by producing death by respiratory failure. If encephalic impulses are blocked by carrying out a section of the medula at the level of the 7th cervial vertebra, with the purpose of having the animal continue with its diaphragmatic respiration the hypertension determined by the citrate injected in the cistern or in the cerebral ventricles is no longer produced (Fig. 3). That is, that when the medular sympathetic centers are not reached, the abdominal vasoconstriction determined by the excitation of the -illegible- and the liberation of adrenaline does not come about.

The bilateral adrenalectomy decreases in a marked way the intensity of the hypertension, which shows the importance of the -illegible- of adrenaline in these phenomena (Fig. 4). The hypertension in the adrenalectomized animals is determined exclusively by the excitation of the endings of the sympathetic. The blocking of the sympathetic by means of high doses of ergotamine does not impede the hypertension determined by the intracisternal injection of the decalcifying substance. (Fig. 5) Likewise, the paralysis of the symphatetic ganglions, which according to -illegible- and Dale (1915) and Acheson and Moe (1945) are produced by the salts of tetraethylammonium, does not change the hypertension curve conditioned by sodium citrate in the nervous centers, in dogs injected with 30 miligrams per kilo weight of said salt (Fig. 6).

Fig. 3.- Lack of pressure response to the injections of sodium citrate in

animals with section of the spinal medula at the level of the 7th cervical vertebra.

Fig. 4. Bilateral adrenalectomy decreases the intensity of the hypertension produced by sodium citrate.

Finally the curarization obtained by means of a raw extract of erythrina crista galli in animals with artificial respiration, although it suppresses the initial abrupt rise of arterial pressure which is observed some seconds after the injection of the sodium citrate, does not impede the arterial hypertension which was recorded in normal animals (Fig. 7).

DISCUSSION

In the same way that the injection of potassium in the cistern or in the cerebral ventricles determines an increase of arterial pressure which, according to Cicardo (1949) is determined by the excitation of the vasomotory centers of the hypothalamus and of the 4th ventricle and of the hypophysis, the injections of decalcifying substances such as citrate, oxalate or phosphate of sodium, on the precipitation of the calcium ion, an antagonistic element to the potassium ion, produces an arterial hypertension with characteristics almost equal to those conditioned by potassium.

The difference which was recorded between the arterial hypertension produced by potassium and by the decalcifying agents, was the lack of intervention of the hypophysis in this last case, since in animals with high section of the medula, the intracisternal injections of citrate do not produce an increase in pressure since only the sympathetic adrenal mechanism would intervene in its production. Fig. 5. Ergotamine does not impede hypertension by intracisternal citrate.

Nervous impulses originated by the decalcification of the vasomotory centersn, on not being able to reach, due to the high section of the medula, the endings of the splanchnics, nor the adrenals, cannot cause the arterial hypertension recorded in the intact animals injected in the same way.

The intervention of the adrenal and sympathetic factors, whose affects add

up are evident since, as has been seen, the bilaterial extirpation of said gland decreases in a marked way arterial hypertension.

The attempts made for even a partial blocking of the sympathetic endings, by means of sympathecholitics such as ergotamine, have yielded fruitless results. In spite of the injection of 2 milligrams of this drug for every 10 kg. of weight, the hypertensory action of the intracisternal sodium citrate, could not be avoided or decreased. Likewise, the tetraethylamonium which blocks, according to some authors,the sympathetic ganglions, does not greatly change the curve of hypertension, in spite of high doses administered.

Curare affects the form of the curve without impeding hypertension. It is evident that the rise in pressure instead of being abrupt, with an initial peak, takes place in a progressive form, reaching vlaues as high as in those of non curarized animals. Therefore, it could be admitted, in accordance with Mullin, Hastings and Lees (1938) that the suppression of muscular somatic activity determines circulatory changes less marked than in those of non curarized animals; however, it must be pointed out that in our experimental conditions, the injections of sodium citrate in the encephalon did not produce generalized contractures, which makes us think that the curarizing agent acts directly on the sympathetic receptors, changing the response.

Fig. 6.- The tetraethylamonium in high dose does not impede hypertension by sodium citrate.

CONCLUSIONS

1. The decrease of the calcium ions of the nervous centers by intracisternal or intraventricular injections of sodium citrate, oxalate or phosphate, produces marked arterial hypertension and respiratory excitation.

Fig. 7.- The curarization with a raw extract of erythrina crista galli impedes the abrupt initial rise but not hypertension.

2. This hypertension would be due to the excitation of the encephalic vasomotory centers and is entirely suppressed after the section of the cervical

medula, which allows assuring that the nervous impulses are propagated to the sympathetic medular centers and the esplanchnics.

3. Bilateral adrenalectomy decreases in a marked form the hypertension, while the sympathecholitic drugs, such as ergotamine or tetraethylamonium salts in high doses do not greatly affect the intensity of hypertension.

4. The repetitions of intracisternal injections of sodium nitrate or the administration of a high dose,produces death by respiratory failure.

Bibliography....follows.

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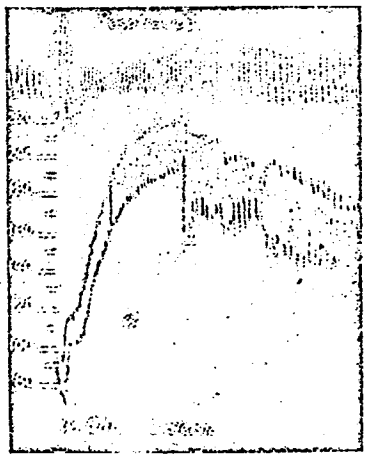

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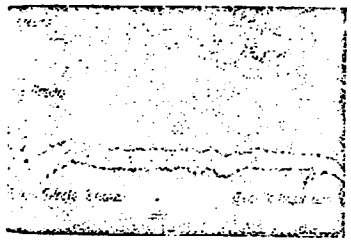

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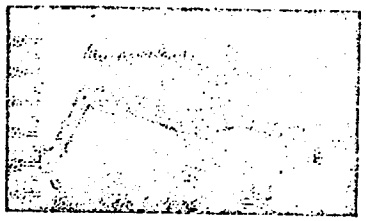

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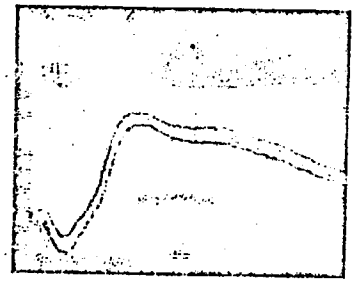

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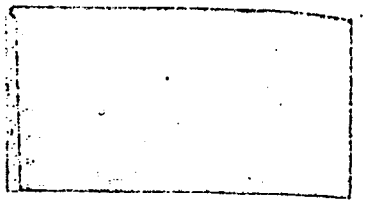

Figure 7

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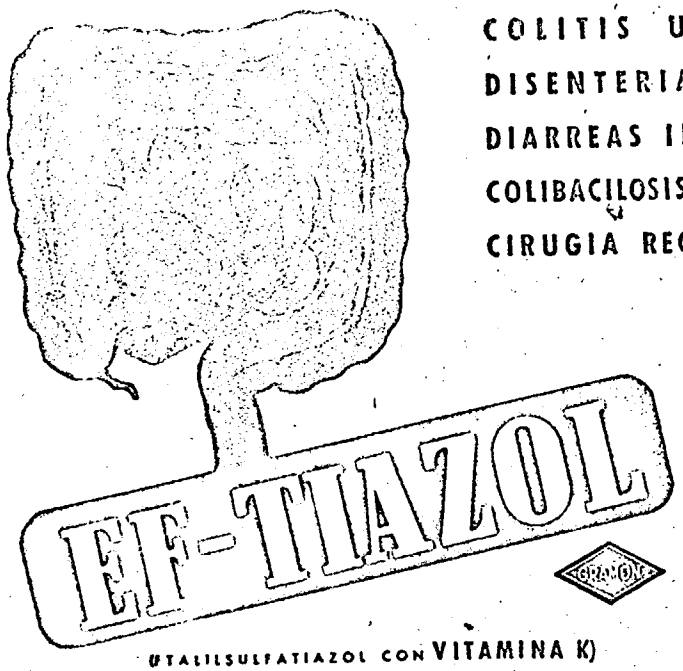

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El FTALILSULFATIAZOL *es el sulfamidado de mayor actividad contra la flora bacteriana intestinal, de acción específica sobre el colibacilo.*

✳

La VITAMINA K *evita la posible hipoprotrombinemia que podría producirse como consecuencia de la disminución de los bacilos coli, factor tan importante en cirugía.*

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ARTICULOS ORIGINALES

LA HIPERTENSION ARTERIAL OBTENIDA POR INYECCIONES CISTERNALES DE CITRATO DE SODIO

Por el Doctor V. H. CICARDO

Profesor titular de Física Biológica de la Fac. de Medicina de Buenos Aires
(Centro de Investigac. Tisiológicas. H. Tornú)

Conocida es la acción excitadora que bre los centros nerviosos producen las ecciones de soluciones isotónicas de clo- ro de potario en el líquido céfalorraquí- o. Estos fenómenos de excitación coin- en con un marcado ascenso de la pre- n arterial y modificaciones respirato- s, según los estudios realizados por rr y Chvoles (1933), Vleeschowver 935), Hermann, Jourdan, Morin y Vial 938), Walker, Smolik y Gilson (1945), ma y Wright (1947), Cicardo (1949), tera.

Fenómenos similares a los producidos r las inyecciones de potasio determina disminución del calcio iónico en los cen- s nerviosos, obtenida ya sea por inyec- nes de citrato de sodio o por la perfu- n de líquidos desprovistos de calcio. obatini (1901) había comprobado que aplicación de soluciones isotónicas de lato o citrato de sodio en el cerebro des- ierto del perro determinaba un au- nto de la excitabilidad del animal y convulsiones generalizadas, las cuales desaparecían con la administración de calcio. Estos mismos hechos han sido observados, también en el perro, con inyecciones intracisternales de citrato de sodio, por Huggins y Hasting (1933) y por Mullin, Hasting y Lees (1938), quienes agregan que además de la excitación neuromuscular se produce estimulación respiratoria y aumento de la presión arterial, provocada principalmente por la contractura de los músculos somáticos, pues se suprime en gran parte por medio del curare. Recientemente, Leusen (1948) ha registrado durante la perfusión de los ventrículos cerebrales de líquidos en los que se aumentaba la concentración de potasio o se suprimía el calcio, hipertensión arterial e hiperexcitabilidad refleja de los vasomotores. Es indudable, de acuerdo con estos hechos experimentales, que la excitación de los centros nerviosos por el aumento de potasio o la disminución del calcio iónico en el líquido céfalorraquídeo,

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produce un aumento acentuado de la presión arterial, que en el caso del citrato de sodio no ha sido bien estudiado ni se ha precisado el mecanismo de producción. Complementando nuestro trabajo anterior en el que se establecía que en la hipertensión producida por las inyecciones de potasio en los centros nerviosos, intervenía el simpático, las adrenales y la hipófisis, procedimos a estudiar el mecanismo de la hipertensión arterial que determinan las inyecciones de citrato de sodio en los centros nerviosos.

METODO

Se utilizaron para la realización de estas experiencias alrededor de 50 perros anestesiados con Nembutal, a los cuales se les registraba la presión arterial a nivel de la arteria femoral y la respiración por medio de un neumógrafo en conexión con una cápsula de Marey.

Se efectuaron las inyecciones de citrato o de oxalato al 3 por ciento en el líquido céfalorraquídeo, preferentemente a nivel de la cisterna magna y en ocasiones en los ventrículos laterales del cerebro o en la base del cerebro, en cantidades que variaban entre 0,1 y 1 centímetro cúbico.

Para establecer el mecanismo de producción de la hipertensión arterial que produce la inyección de citrato en los centros nerviosos, se estudió la intervención y el comportamiento del simpático, de las adrenales y de la contractura muscular. Con el objeto de excluir los centros simpáticos medulares se hicieron experiencias con animales a los cuales se les seccionaba la medula espinal a la altura de la 7ª vértebra cervical.

También se estudió las características de la hipertensión obtenida por inyecciones intracisternales de citrato de sodio después del bloqueo del simpático, con 1 ó 2 miligramos de Ergotamina o bien después de la inyección de 30 miligramos por kilo de peso de cloruro de tetraetilamonio que determina, según algunos autores, el bloqueo de los ganglios del sistema nervioso autónomo en la misma forma que la nicotina.

La adrenalectomía bilateral por vía abdominal se efectuó en algunos animales con el objeto de precisar el papel que desempeña esta glándula en este tipo de hipertensión nerviosa.

Para descartar la intervención de la contractura neuromuscular que produce el citrato, aun en muy poca intensidad en nuestras condiciones experimentales, se hicieron inyecciones intracisternales en animales totalmente curarizados, por medio de un extracto bruto de *erythrina crista galli*, a quienes se les mantenía con respiración artificial. Finalmente en algunos animales las experiencias se realizaron después de la resección de los nervios vagos.

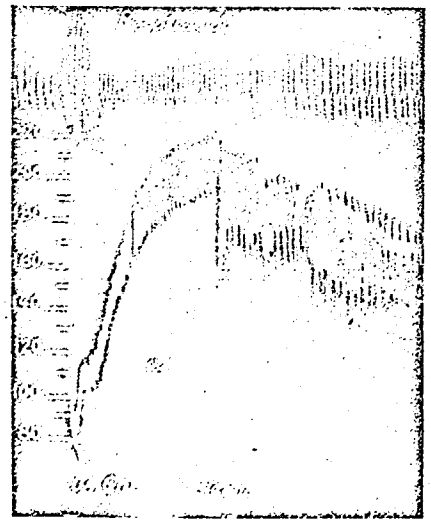


FIGURA 1. — *Modificaciones de la presión arterial y de la respiración por inyecciones intracisternales de citrato de sodio.*

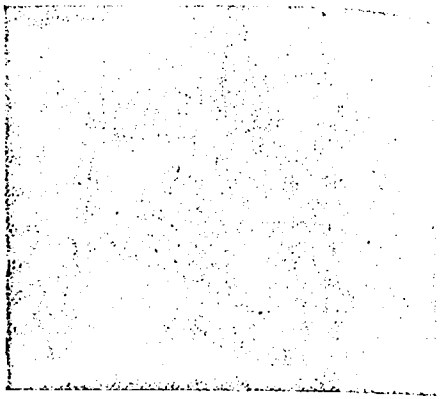
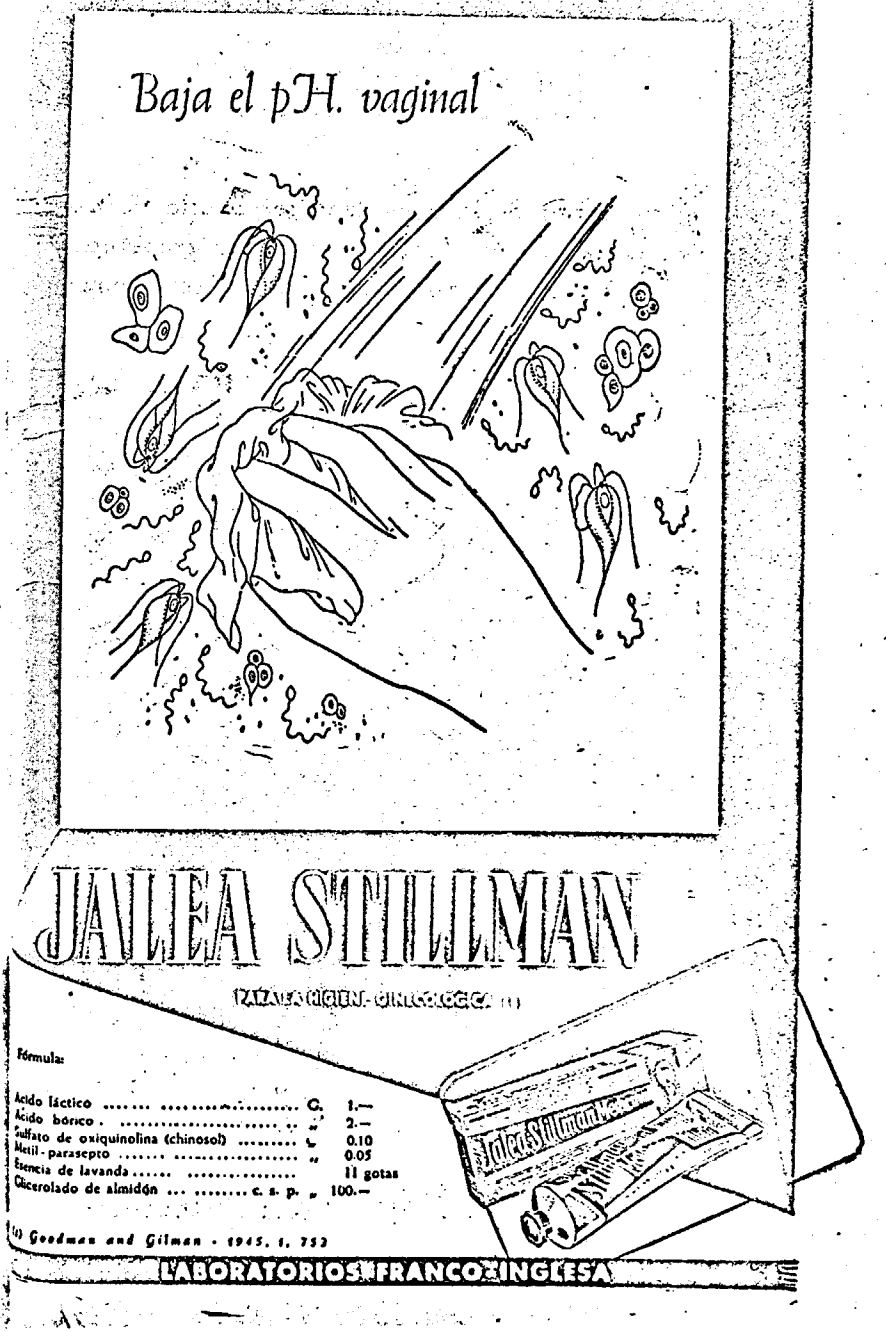


FIGURA 2. — *Modificaciones de la presión y de la respiración por inyecciones de citrato de sodio en el hipotálamo.*

RESULTADOS

La hipertensión que produce el citrato de sodio intracisternal así como en los ventrículos laterales o en el mismo espesor del hipotálamo es marcada pero transitoria (Figs. 1 y 2).

El aumento de presión puede llegar a 10 centímetros de mercurio y más, inmediatamente después de la inyección, para mantenerse luego unos minutos y descender progresivamente alcanzando su valor inicial alrededor de los 15 minutos. En algunos casos la presión se mantiene algo más elevada que al principio de la experiencia. Al mismo tiempo que se inicia el aumento de



Medicación espasmolítica

Trasentina

Clorhidrato del éster dietilaminoelítico del ácido difenilacético.

Antiespasmódico sintético de acción miógena y neurógena.

Ampollas *Grageas* *Supositorios*

Espasmo-Cibalena

Trasentina + Cibalena

Antiespasmódico y analgésico sin alcaloides.

Grageas *Supositorios* *(niños y adultos)*

Neuro-Trasentina

Trasentina + Ac. feniletilbarbitúrico.

Espasmosedativo y somnífero.

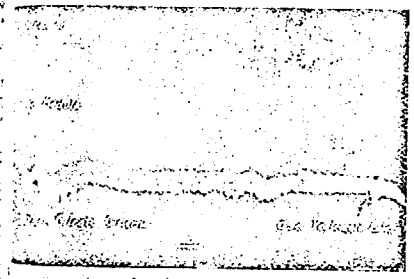
Grageas

PRODUCTOS QUIMICOS CIBA S. A.

...sión se registra por lo general un ligero paro ...piratorio seguido de un aumento de la ampli... y de la frecuencia respiratoria. Si la canti... de citrato inyectada es algo elevada, sobre...e el paro respiratorio y la muerte, que puede ...arse con la respiración artificial. Este paro ...piratorio se produce más fácilmente, o sea ... dosis más bajas, cuando las inyecciones son ...ernales que cuando se efectúan en las otras ...as del líquido céfalorraquídeo. Este hecho se ...lica por la proximidad del centro respirato... que se inhibe más fácilmente al ser alcan...o por mayores concentraciones de citrato.

Si se bloquean los impulsos encefálicos efectuan...itación de los centros vasomotores o respira...ios se producen en menor intensidad y los ...mentos son cada vez menores y terminan por ... producirse por la intoxicación de dichos cen...s. Además, la repetición de una dosis termi... por producir la muerte por paro respiratorio.

Si bloquean los impulsos encefálicos efectuan... una *sección de la medula* a la altura de la 7ª ...tebra cervical, con el objeto de que el ani...al continúe con su respiración diafragmática,



...GURA 3. — *Falta de respuesta presora a las ...yecciones de citrato de sodio en animales con ...ción de la medula espinal a la altura de la 7ª vértebra cervical.*

... hipertensión que determina el citrato inyec...o en la cisterna o en los ventrículos cerebra..., ya no se produce (Fig. 3). Es decir, que ... no ser alcanzados los centros simpáticos me...ares, no sobreviene la vasoconstricción abdo...nal que determina la excitación de los esplác...os y la liberación de adrenalina.

La *adrenalectomía bilateral* disminuye en for... acentuada la intensidad de la hipertensión, ... que demuestra la importancia que tiene la ...ración de adrenalina en estos fenómenos (Fig. ... La hipertensión en los animales adrenalec...izados, está determinada exclusivamente por ... excitación de las terminaciones del simpático.

El bloqueo del simpático por medio de dosis ...as de *ergotamina* no impide la hipertensión ... determina la inyección intracisternal de la ...stancia descalcificante (Fig. 5). Igualmente, ... parálisis de los ganglios simpáticos, que según ...n y Dale (1915) y Acheson y Moe (1945) ...ducen las *sales de tetraetilamonio*, no modi... la curva de la hipertensión condicionada ... el citrato de sodio en los centros nerviosos,

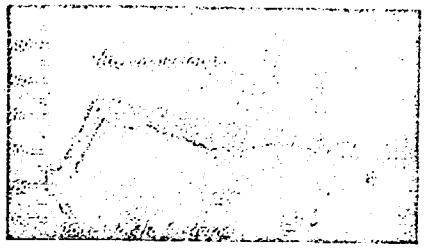


FIGURA 4. — *La adrenalectomía bilateral disminuye la intensidad de la hipertensión producida por el citrato de sodio.*

en perros inyectados con 30 miligramos por kilo de peso de dicha sal (Fig. 6).

Finalmente, la *curarización* obtenida por medio de un extracto bruto de *erythrina crista galli* en animales con respiración artificial, si bien suprime el ascenso brusco inicial de la presión arterial que se observa algunos segundos después de la inyección del citrato de sodio, no impide la hipertensión arterial que se registra en los animales normales (Fig. 7).

DISCUSION

De la misma manera que la inyección de potasio en la cisterna o en los ventrículos cerebrales determina un aumento de la presión arterial, que según Cicardo (1949) está determinada por la excitación de los centros vasomotores del hipotálamo y del 4º ventrículo y de la hipófisis, las inyecciones de substancias descalcificantes como el citrato, oxalato o fosfato de sodio, al precipitar el ión calcio, elemento antagónico del ión potasio, produce una hipertensión arterial con características casi iguales a las que condiciona el potasio.

La diferencia que se registró entre la hipertensión arterial producida por el potasio y por los agentes descalcificantes, fué la falta de la intervención de la hipófisis en este último caso, pues en los animales con sección alta de la medula, las inyecciones intracisternales de citrato no producen aumento de presión, debido a que

FIGURA 5. — *La ergotamina no impide la hipertensión por el citrato intracisternal.*

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solamente el mecanismo simpático adrenal intervendría en su producción.

Los impulsos nerviosos originados por la descalcificación de los centros vasomotores, al no poder alcanzar, por la sección alta de la medula, las terminaciones de los esplácnicos, ni las adrenales, no puede causar la hipertensión arterial registrada en los animales intactos inyectados de la misma manera.

Es evidente la intervención de los factores adrenales y simpáticos cuyos efectos se suman, pues, según se ha visto, la extirpación bilateral de dicha glándula disminuye en forma marcada la hipertensión arterial.

Las tentativas realizadas para un bloqueo aun parcial de las terminaciones simpáticas, por intermedio de simpáticolíticos como la ergotamina, han dado resultados infructuosos. No obstante la inyección de 2 miligramos de esta droga por cada 10 kg. de peso, la acción hipertensora del citrato de sodio intracisternal, no pudo evitarse ni disminuirse. Igualmente el tetraetilamonio que bloquea, según algunos autores, los ganglios simpáticos, no modifica mayormente la curva de la hipertensión, a pesar de las altas dosis administradas.

El curare afecta la forma de la curva sin impedir la hipertensión. Es evidente que el ascenso de la presión en lugar de ser brusco, con un pico inicial, se hace en forma progresiva, alcanzando valores tan altos como en los animales no curarizados. Por lo tanto podría admitirse, de acuerdo con Mullin, Hastings y Lees (1938) que la supresión de la actividad somática muscular determina cambios circulatorios menos marcados que en los no curarizados, pero debe señalarse que en nuestras condiciones experimentales, las inyecciones de citrato de sodio en el encéfalo no producían contracturas generalizadas, lo que nos hace pensar que el agente curarizante actúa directamente sobre los receptores simpáticos, modificando la respuesta.

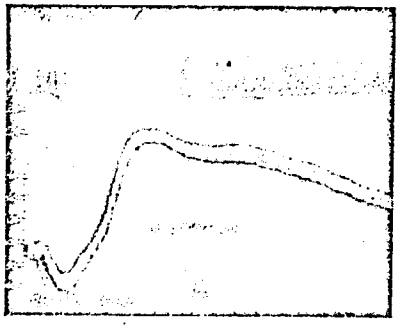

FIGURA 6. — *El tetraetilamonio en dosis elevada no impide la hipertensión por el citrato de sodio.*

CONCLUSIONES

1º La disminución del ión calcio de los centros nerviosos por inyecciones intracisternales o intraventriculares del citrato, oxalato o fosfato de sodio, produce marcada hipertensión arterial y excitación respiratoria.

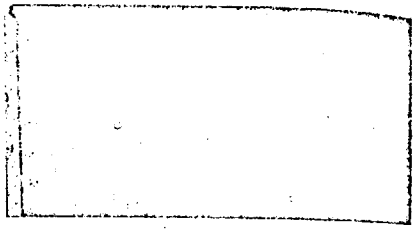

FIGURA 7. — *La curarización con un extracto bruto de* erythrina crista galli *impide el aumento brusco inicial pero no la hipertensión.*

2º Esta hipertensión sería debida a la excitación de los centros vasomotores encefálicos y se suprime totalmente después de la sección de la medula cervical, lo que permite asegurar que los impulsos nerviosos se propagan a los centros simpáticos medulares y a los esplácnicos.

3º La adrenalectomía bilateral disminuye en forma acentuada la hipertensión, mientras que las drogas simpáticolíticas, como la ergotamina o sales de tetraetilamonio en altas dosis no afectan mayormente la intensidad de la hipertensión.

4º Las repeticiones de las inyecciones intracisternales de citrato de sodio o la administración de una dosis elevada, produce la muerte por paro respiratorio.

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Arch. int. Pharmacodyn., 1950, LXXXIII, No 1.

LABORATORIO DE BIOFISICA DE LA FACULTAD DE CIENCIAS MÉDICAS
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ARTERIAL HYPERTENSION OBTAINED BY CISTERNAL INJECTIONS OF SODIUM CITRATE

BY

V. H. CICARDO

(Received for publication 2-12-1949).

The exciting action which the injections of isotonic potassium chloride solutions into the cephalorachidian liquid produce on the nerve centers, is well known. These exciting phenomena coincide with a marked increase in arterial pressure and respiration rate, according to the studies realized by STERN & CHOVLES (1933), DE VLEESCHHOUWER (1935), HERMANN, JOURDAN, MORIN & VIAL (1938), WALKER, SMOLIK & GILSON (1945), CALMA & WRIGHT (1947), CICARDO (1949), etc...

Similar phenomena to those produced by potassium injections are determine by decrease of ionic calcium in the nerve centers, which is obtained, either by sodium citrate injections or by perfusion of calcium free liquids. SABBATINI (1901) has proved that the application of isotonic solutions of oxalate or sodium citrate into the open brain of the dog caused an increase of the animal's excitability and general convulsions which disappeared with the administration of calcium. HUGGINS & HASTINGS (1933) and MULLIN, HASTING & LEE (1938) observed the same facts also in dogs with intracisternal injections of sodium citrate, and they added that, besides the neuromuscular excitation, respiratory stimulation and increase in arterial pressure were produced, principally through the contracture of the somatic muscles, and then eliminated to a great extent by curare. Recently, LEUSEN (1948) has observed during perfusion of the cerebral ventricles with solutions containing an elevated potassium concentration or without calcium, arterial hypertension and reflex hyperexcitability of the vasomotorcenter. Undoubtedly, it is in accordance with these experimental facts that the excitation of the nerve centers, due to the increase of potassium or the decrease of ionic

calcium in the cephalo
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calcium in the cephalorachidian liquid, produces an important rise in arterial pressure, which, in the case of sodium citrate, has not been well studied so far, nor has the mechanism been established. In addition to our previous work in which we reported that the hypertension produced by potassium injections in the nerve centers, intervened the sympathetic, the adrenals and the hypophysis, we proceed to study the mechanism of arterial hypertension which is produced by sodium citrate injections into the nerve centers.

METHODS

For these experiments we have used about 50 dogs, anaesthetized with Nembutal, in which were registered arterial pressure at the level of the femoral artery, and respiration by means of a pneumograph in connection with a Marey tambour.

Injections of citrate, oxalate or sodium phosphate at 3 per cent were administered into the cephalorachidian liquid, preferably at the level of the cisterna magna, and occasionally into the lateral ventricles of the brain or in the base of the brain, in quantities varying between 0.1 cc. and 1 cc.

To investigate mechanism of the arterial hypertension which is produced by citrate injections into the nerve centers, the intervention and behaviour of the sympathetic, the adrenals and the muscular contracture were studied. In order to exclude the medullary sympathetic centers, experiments were carried out with animals, in which the spinal cords were severed at the 7th cervical vertebra.

Also the characteristics of the hypertension obtained by intracisternal injections of sodium citrate after blocking of the sympathetic with 1 or 2 mg. of ergotamine, or after injecting 30 mg. per kilo of weight of tetrathylamonium chloride, were studied, which, according to some authors, the blocking of the ganglia of the autonom nervous system establishes in the same way as nicotine does.

The bilateral adrenalectomy via the abdomen was carried out in some animals with the object of defining the action of this gland in this type of nervous hypertension.

To eliminate the intervention of the neuromuscular contracture caused by citrate, intracisternal injections were given to animals totally curarized, by means of « Erythrina crista galli » extract, continuing to give them artificial respiration. Finally, in some animals these experiments were carried out after resection of the vagus nerves.

Results

The hypertension produced by intracisternal sodium citrate, either in to the lateral ventricles or in to the hypothalamus itself, is marked but transitory. (Fig. 1 and fig. 2).

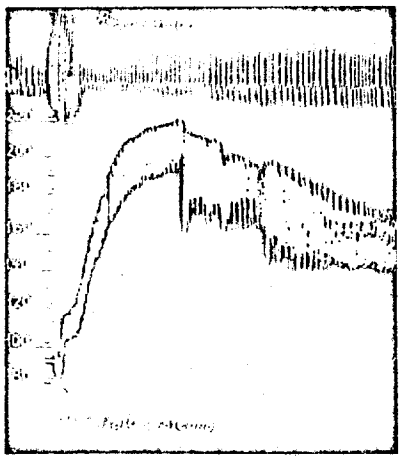

Fig. 1
Modifications of arterial pressure and respiration caused by intracisternal injections of sodium citrate.

The rise in pressure may reach 10 centimeters of mercury or more immediately after the injections, remaining there for some minutes and decreasing progressively, finally reaching its initial value in about 30 minutes. In some cases, the pressure is maintained somewhat higher than at the beginning of the experiment. Simultaneously with the rise in pressure, a slight pause in respiration generally occurs, followed by an increase of respiratory amplitude and frequency. If the quantity of injected citrate is somewhat high, breathing stops and death occurs, which can be prevented by artificial respiration. This cessation of breathing occurs more promptly when lower doses

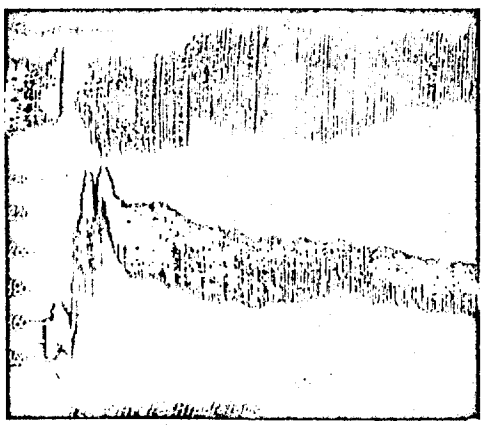

Fig. 2
Modifications of pressure and respiration caused by sodium citrate injections into the hypothalamus.

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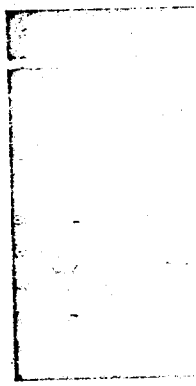

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are administered as the injections are cisternal, than when they are given in other zones of the cephalorachidian liquid. This is explained by the proximity of the respiratory center which is more easily inhibited when reached with higher citrate concentrations.

With repeated injections, the excitation of the vasomotor or respiratory centers occur with less intensity and the stimulations are

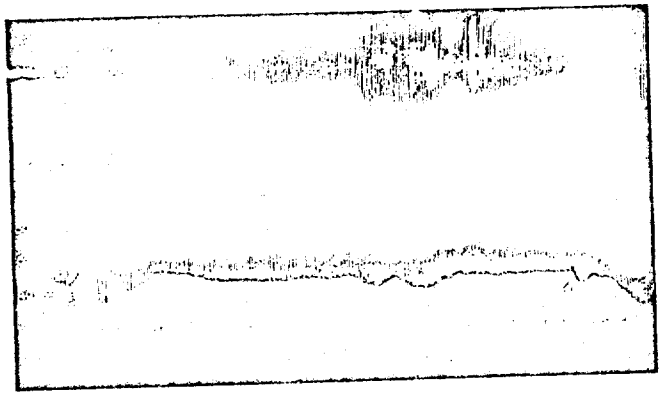

FIG. 3

Lack of hypertension to sodium citrate injections in animals with severing of the spinal cord at the height of the 7th cervical vertebra.

smaller each time, until they stop completely, due to intoxication of these centers. Moreover, the repetition of one dose finally causes death due to cessation of breathing.

When the encephalic impulses are blocked by a *severing of the spinal cord* at the height of the 7th cervical vertebra, so that the animal may continue its diaphragmatic breathing, the hypertension produced

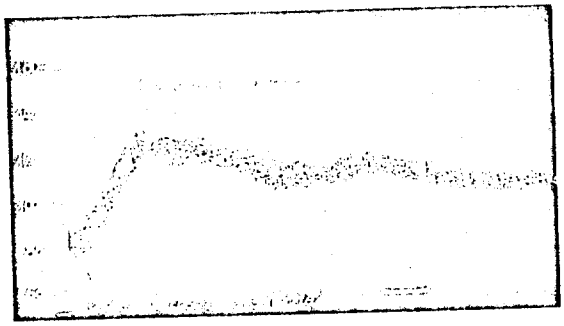

FIG. 4

Bilateral adrenalectomy diminished the hypertension intensity produced by sodium citrate.

by the citrate injected in the cistern or in the cerebral ventricles, does not occur any more (Fig. 3). This means, that when the spinal sympathetic centers are not reached, abdominal vasoconstriction is not produced by lack of excitation of the splanchnic nerves and liberation of adrenaline.

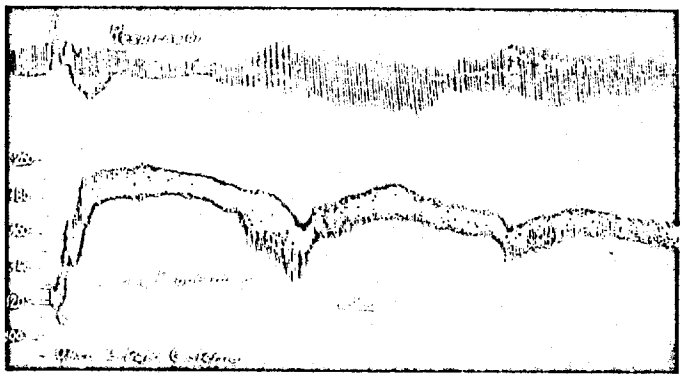

FIG. 5
Ergotamine does not prevent the hypertension by intracisternal citrate.

Bilateral *adrenalectomy* diminishes considerably the intensity of hypertension, which proves the importance of the liberation of the adrenaline in these phenomena. Fig. 4. The hypertension of adrenalectomized animals is caused exclusively by excitation of the sympathetic endings.

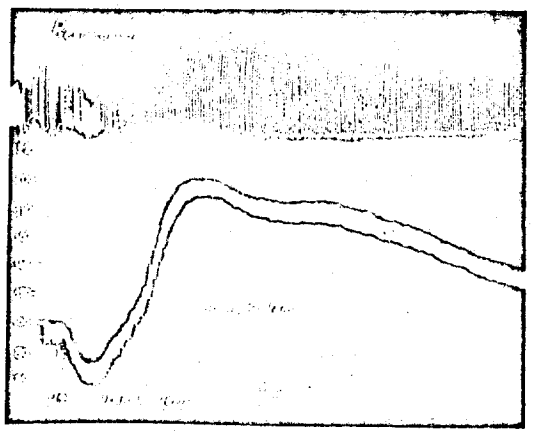

FIG. 6
Tetraethylammonium in increased doses does not impide the hypertension by sodium citrate.

The blockage of the sympathetic by means of high doses of *ergotamine* does not prevent hypertension caused by the intracisternal injection of the decalcifying substance (Fig. 5). Equally, paralysis of the sympathetic ganglia, which according to BURN and DALE (1915) and ACHESON and MOE (1945) is produced by the *tetraethylammonium salts*, does not modify the hypertension caused by the sodium citrate in the nerve centers, in dogs injected with 30 mg. per kilo of weight of the said salt. Fig. 6.

Finally the *curarization* obtained by means of « Erythrina crista galli » extract in dogs with artificial respiration, although it suppresses the initial sudden rise in arterial pressure, which is observed a few seconds after the sodium citrate injection, does not prevent the arterial hypertension, reported in normal animals (Fig. 7).

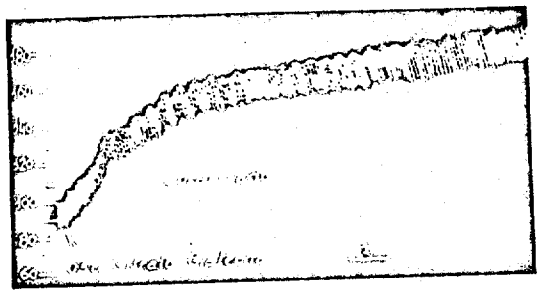

FIG. 7

Curarization with an " Erythrina crista galli " extract prevents the sudden initial rise, but not the hypertension.

DISCUSSION

The potassium injections into the cistern or into the cerebral ventricles produce an increase in arterial pressure, which, according to CICARDO (1949), is caused by the excitation of the vasomotor centers of the hypothalamus and of the 4th ventricle and of the hypophysis. Injections of decalcifying substances such as citrate, oxalate or sodium phosphate, which precipitate the calcium ion, produce an arterial hypertension with the same characteristics as those induced by potassium.

The difference between arterial hypertension produced by potassium and by the decalcifying agents, is the lack of intervention of the hypophysis in this last case, for, in animals with a high severing of the spinal cord, the intracisternal citrate injections do not cause a rise in pressure, thas showing that only the sympathetic adrenal mechanism plays a role in its production.

The nerve impulses induced by the decalcifying of the vasomotor centers cannot produce arterial hypertension, reported in intact animals injected in the same way, when they do not reach the splanchnic nerve endings, nor the adrenals due to the high severing of the spinal cord.

The intervention of the adrenal and sympathetic factors is evident, uniting their efforts, for, as has been observed, the bilateral extirpation of this gland decreases remarkably the arterial hypertension.

Experiments carried out for the blockage, even partial, of the sympathetic endings through sympatholytics, such as ergotamine, have given fruitless results. Nevertheless, it is not possible to prevent or diminish the hypertension action of the intracisternal sodium citrate with the injection of 2 mg. of this drug per 10 kilo of body weight. Nor does the tetraethylammonium which, according to some authors, blocks the sympathetic ganglia, modify the hypertension greatly, in spite of the high doses administered.

Curare influences the line of the curb without preventing hypertension. It is evident, that the rise in pressure, instead of being sudden with an initial peak, is done in a progressive form and reaches such high values as in non-curarized animals. Therefore, it can be admitted, agreeing with MULLIN, HASTINGS and LEE (1938), that the suppression of the somatic muscular activity effects circulatory changes less than in non-curarized. But it must be noted that in our experimental conditions, sodium citrate injections in the encephalon do not produce general contracture, which makes us think that the curarizing agent acts directly on the sympathetic receptor modifying the response.

SUMMARY

1) The decrease in calcium ion of the nerve centers by intracisternal or intraventricular injections of citrate, oxalate or sodium phosphate produces a marked arterial hypertension and respiratory excitation.

2) This hypertension would be due to the excitation of the vasomotor centers and is completely eliminated after section of the cervical spinal cord, which assures that the nervous impulses are diffused to the spinal sympathetic centers.

3) Bilateral adrenalectomy decreases considerably the hypertension, while sympatholytic drugs, such as ergotamine or tetraethylammonium salts in high doses do not affect greatly hypertension intensity.

4) Neuromuscular curarization does not prevent the hypertension, it only suppresses the sudden initial rise.

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5) Repetitions of intracisternal sodium citrate injections or the administration of an increased dose, produce death from cessation of respiration.

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destillata are, however, considerably higher. We cannot offer any elucidation since the endosmotic afflux does not seem a satisfactory explanation.

In principle it is of no consequence, which of the described test arrangements is used for the continuous determination of calcium in whole-blood. We prefer the single dialysis since double dialysis requires considerably more time and equipment. As the error margin is greater when dialysing against calcium-free Ringer's solution, we shall in future dialyse against aqua destillata.

By using the described method in animal experiments we are able to show fluctuations in the calcium ion concentration. We do not maintain, however, that all the calcium measured is ionised *in vivo*.

Summary

The continuous fluorometric determination of calcium in whole-blood is possible after continuous dialysis. As calcium is present in blood in various forms, several possibilities exist for varying dialysis time and liquids used in counter-dialysis. The results obtained by this method are discussed in this paper.

Zusammenfassung

Kontinuierliche fluorometrische Bestimmung von Calcium in Vollblut und Plasma

Nach fortlaufender Dialyse ist eine kontinuierliche fluorometrische Bestimmung von Calcium in Vollblut möglich. Da Calcium im Blut in verschiedenen Zustandsformen vorliegt, ergeben sich durch Veränderung der Dialysezeiten und der in der Gegendialyse verwendeten Flüssigkeiten verschiedene Variationsmöglichkeiten. Die auf diese Weise ermittelten Ergebnisse werden diskutiert.

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Aus der Abteilung für experimentelle Therapie der Universität Freiburg i. Br.; Vorstand: Professor Dr. med. P. Marquardt

The Continuous Determination of Calcium and Potassium in the Whole-Blood of Cats after an Infusion of Sodium Citrate*)

By H. G. Claßen, P. Marquardt and M. Späth

The toxicity of sodium citrate was revealed by the experiments of Mitscherlich in 1845 [1]. Tetanoid convulsions and circulatory failure frequently occur after intravenous, subcutaneous or even peroral administration [2]. Sabatini [3] and Loeb [4] explained this effect by a decrease in the calcium ion concentration in the blood. Animal experiments proved that the toxicity of an infusion of sodium citrate depends not only on the total concentration administered but also on the citrate dose per unit of time and on the type of test animal.

Cats, for instance, are more sensitive than rabbits [5]. The human compatibility towards citrate per unit of time has been frequently discussed since citrate was first used in preserved whole-blood (1.9 g/500 ml) [6, 7, 8, 9, 10, 11]. The question as to how citrate is tolerated arises also, for instance, when it is injected intravenously, for instance when syringes for blood tests are exchanged in error [12], when it is administered perorally for the alkalisation of urine or in the case of lead or uranium poisoning [13]. According to Kuschinsky [14] 1 mg sodium citrate/kg/min administered i.v. is harmless to human beings.

As was suspected very early, calcium forms a soluble, undissociated complex with citrate (for details see [15]). The original investigations were carried out by Hastings and coworkers [16]. According to the team's results tertiary calcium citrate dissociates in two stages: the first stage is almost completely dissociated like a strong electrolyte, the second stage is only slightly dissociated like a weak electrolyte. The concentration of calcium ions in a calcium citrate solution is equal to or exceeds one third of the total calcium content [16].

1. $Ca_3Cit_2 \rightleftharpoons Ca(CaCit)_2 \rightleftharpoons Ca^{++} + 2\,CaCit^-$
2. $CaCit^- \rightleftharpoons Ca^{++} + Cit^{---}$

Citrate is present in the human whole-blood in concentration of 1.5 to 4 mg%; 0.2—1.0 g/day is excreted in urine [17]. The soluble undissociated calcium complex is consequently important in the calcium metabolism, and has been proved in the acid and the alkaline medium [18]. Its function as an intermediary between the insoluble calcium deposits in the bones and the calcium in the blood serum comes to mind, as well as being a form of calcium that can be excreted in urine. A decrease in non-diffusible calcium in favour of diffusible calcium and an increase in the total calcium content of the blood was established in dogs after administration of sodium citrate; the calcium excretion in urine increased simultaneously [17].

Sodium citrate intoxication seemed, therefore, suitable as a means of testing the method suggested by us for the continuous determination of calcium. After dialysis, only that portion of the total calcium is present in the dialysate that is dialysable at this moment. Only the calcium ions together with calcein form a complex whose fluorescence is measured. Therefore, a fall in the curve may be expected after administration of an effective dose of sodium citrate. This means that the concentration of calcium ions decreases with constant or increasing total calcium content.

Method

Cats of 3.0 kg body weight in hexobarbital narcosis were used as test animals. They received an i.v. dose of 3000 units by weight/kg heparin[1]) to inhibit blood coagulation. A tracheotomy was performed on the animals for the purpose of induced respiration[2]). A cannula was inserted in a femoral vein for injections and infusions[3]).

The following phenomena were registered on recorders [4, 5]):

1. The calcium content of whole-blood after simple dialysis against aqua destillata [19];
2. The potassium content of whole-blood [20];
3. Arterial pressure and pulse rate: the pressure was measured with a Statham-Element P 23 Db in a femoral artery. The measured va-

*) Part of the results was presented at the Joint Summer Meeting of the British and German Pharmacological Societies, Cambridge, 6—9 September, 1967.

[1]) Liquemin®; Manufacturer: Deutsche Hoffmann-La Roche AG., Grenzach in Baden. We are obliged for samples of this product which was made available to us for our experiments.
[2]) Schuler aspirator pump, manufactured by B. Braun, Melsungen (West Germany).
[3]) Infusion pump, manufactured by B. Braun, Melsungen (West Germany).
[4]) Manufactured by Rikadenki, Tokyo (Japan).
[5]) Manufactured by Bristol's Instruments Co. Ltd., London (England).

lines were amplified with a D.C. bridge[6]). The pressure curve was directly recorded. A ratemeter[7]) was connected for recording the pulse rate.

4. The venous pressure in the right auricle of the heart was measured and recorded as in 3.
5. Nerve/muscle preparation: The tibialis nerve was subjected to automatic, time-controlled, intermittent electric stimulation (train): intensity of stimulation: 0.8—1.8 V; stimulation period: 30 msec; basic rhythm: 40 msec (25 Hz); length of series: 3 sec; rhythm of series: 60 sec. A high frequency stimulus unit was used for insulating the stimulus[8]). The contractions of the gastrocnemius muscle were converted into electrical signals with a force transducer[9]) or [10]).
6. The ECG was recorded with a direct-writing ink-oscillograph[11]), paper feed 100 mm/sec.

We are indebted to Mr. E. Schöffel for his valuable technical assistance.

The following substances were injected or infused:

1. Isoprenaline[12]);
2. Calcium Hausmann®[13]), 5 ml = 250 mg calcium in the form of the complex salt of ethylenediamine tetraacetic acid;
3. Calcium Sandoz®[14]), 10 ml = 1.375 g calcium lactobionate = 10% calcium gluconate;
4. Sodium citrate: trisodium citrate —5 1/2-hydrate[15]);
5. Phentolamine[16]).

Results

Initially, increasing amounts of sodium citrate were added to aqueous calcium carbonate solutions and the fluorescence after dialysis was determined: the fluorescence decreases and the curve (see below) declines. As expected, the calcium ions form an undissociated complex with citrate. The mean values of five corresponding tests yielded the curve in Fig. 1.

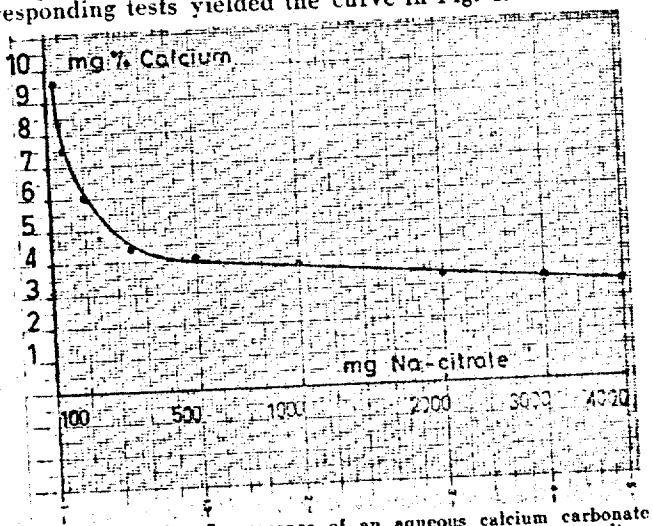

Fig. 1: Decreasing fluorescence of an aqueous calcium carbonate solution as a consequence of increasing amounts of sodium citrate.

The asymptotic course of the curve was to be expected from the findings of Hastings and coworkers: the concentration of calcium ions in a calcium citrate solution must not drop below a certain marginal value if the solubility is to be maintained. In animal experiments only a deficiency in calcium ions can be established under these conditions.

The administration of a sufficiently large dose per unit of time is, of course, a prerequisite. It was, therefore, not surprising that no change in any of the recorded processes was registered in spontaneously breathing cats which had been given 10 mg/min sodium citrate, even after an infusion period of 3 h, *i.e.* after 1.8 g sodium citrate had been administered. A dose of 10 mg/min sodium citrate is, therefore, tolerated by healthy cats.

We then increased the dose to 50 mg/min sodium citrate in 0.5 ml liquid. Symptoms of toxicity now become rapidly manifest (Fig. 2): blood pressure and calcium ion concentration simultaneously drop sharply. Respiration ceases after only a few minutes. A short rise in the blood potassium curve is manifest but can be inhibited by preliminary administration of a sympathicolyticum phentolamine (Regitin®) (Fig. 3) and may thus be regarded as an emergency function. At last the well-known final rise in the potassium [21] and

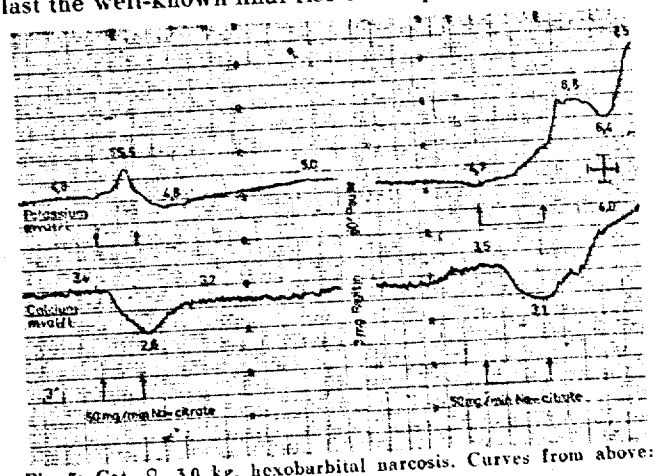

Fig. 3: Cat, ♀, 3,0 kg, hexobarbital narcosis. Curves from above: potassium; calcium.

in the calcium concentration becomes manifest. The contractions of the gastrocnemius muscle following electrical stimulation of the tibialis nerve are pronounced at first owing to the relative increase in potassium ions [22] but become weaker later on. The reason for this may be found in insufficient circulation. The occurrence of tetanoid convulsions was dependent on the depth of narcosis.

The decrease in the calcium content amounted on the average to 1.9 mg% after 10 min and the administration of a total quantity of 500 mg sodium citrate (standard value for calcium in the whole-blood of cats after single dialysis against aqua destillata: 6.90 ± 0.45 mg% [19]).

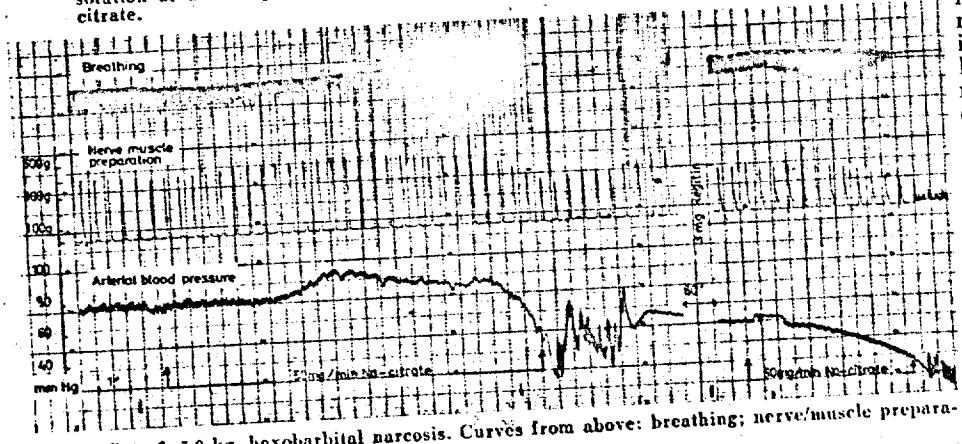

Fig. 2: Cat, ♂, 3,0 kg, hexobarbital narcosis. Curves from above: breathing; nerve/muscle preparation; arterial blood pressure.

[6]) Manufactured by Hugo Sachs Elektronik, Buchheim near Freiburg (West Germany).
[7]) EKA-Puls-Gerät, manufactured by Hugo Sachs Elektronik, Buchheim near Freiburg (West Germany).
[8]) Stimulator II with automatic time control unit and insulating unit manufactured by H. Sachs Electronik, Buchheim near Freiburg (West Germany).
[9]) Grass Transducer FT 03C, manufactured by Grass Medical Instruments, Quincy, Mass., (U.S.A.).
[10]) Force-Transducer, manufactured by Hugo Sachs Elektronik, Buchheim near Freiburg (West Germany).
[11]) Oscillowink, manufactured by Siemens & Halske AG., Karlsruhe (West Germany); fitted with pre-amplifiers, manufactured by Hugo Sachs Elektronik, Buchheim near Freiburg (West Germany).
[12]) N-Isopropyl-noradrenaline-sulfate; Aludrin®; manufacturer: C. H. Boehringer Sohn, Ingelheim a. Rh. (West Germany). We are grateful for trial quantities of the product that were made available for us.
[13]) Manufacturer: Hausmann AG., St. Gallen (Switzerland).
[14]) Manufacturer: Sandoz AG., Nuremberg (West Germany).
[15]) Manufacturer: E. Merck AG., Darmstadt (West Germany).
[16]) 2-(N-p-tolyl-N-m-hydroxyphenyl-aminomethyl)-imidazoline · HCl or methane sulfonic acid, Regitin®; manufacturer: Ciba AG., Basle (Switzerland).

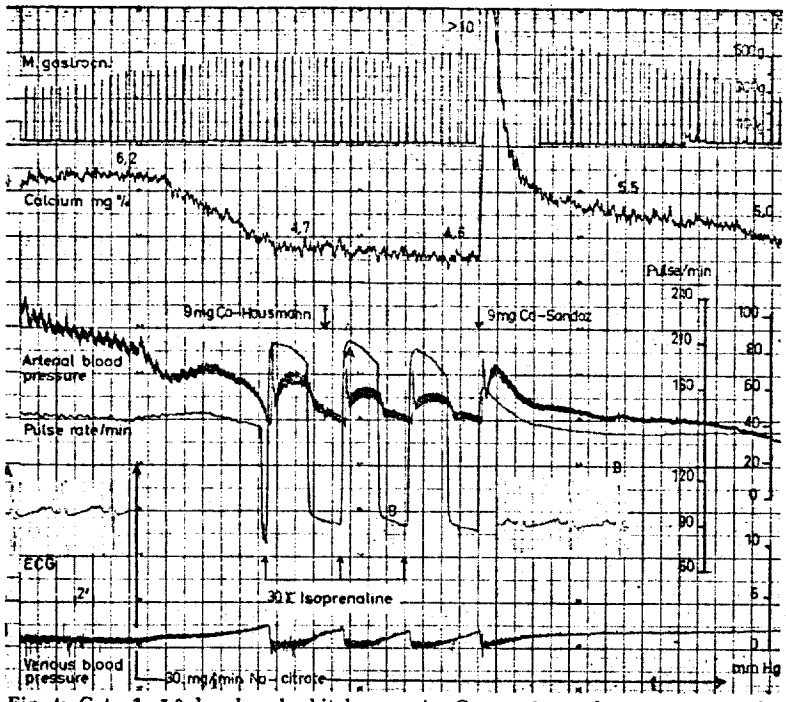

Fig. 4: Cat, ♂, 3.0 kg, hexobarbital narcosis. Curves from above: nerve/muscle preparation; calcium; arterial blood pressure; pulse rate; ECG; venous blood pressure. The top curve must be imagined shifted 5 squares to the right.

At a rate of infusion of only 30 mg sodium citrate per minute in 0.5 ml liquid and uniform induced respiration, the critical drop in blood pressure occurs after a longer latency, on the average after 20 min (see Fig. 4). The QT deflection in the ECG, however, is measurably prolonged at unaltered pulse rate after only 2 to 3 min, *i.e.* at a point when the calcium ion concentration is only slightly reduced (on the average by 0.2 mg%). This retarded excitation regression generally with an isoelectric interval and unmodified T-wave is typical of a calcium ion deficiency [23]. A little later the contractions of the nerve/muscle preparation become more pronounced, as in the preceding test series. As can be seen from Fig. 5, the course of the potassium curve is unaltered. This points to a relative potassium deficiency [22].

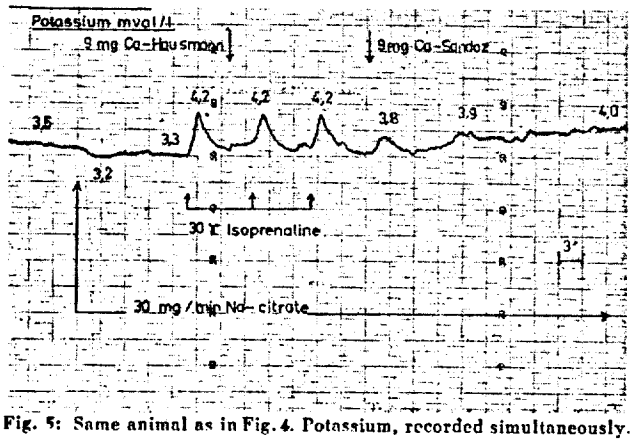

Fig. 5: Same animal as in Fig. 4. Potassium, recorded simultaneously.

If the citrate infusion is carried on, the calcium ion concentration drops further and approaches a marginal value. The circulation is now affected (Fig. 4): The pressure in the arterial system decreases gradually with constant or only slightly reduced pulse rate while the venous pressure rises. The pulse rate then falls rapidly: continued citrate infusion would now lead to *exitus* quite soon. In ten animals this point was reached after an average of 20 min and a decrease in the calcium of about 1.4 mg%. Nevertheless the RR distance remains almost the same in the ECG, indicating that the mechanical mechanism is severely damaged and causes severe insufficiency while the electric heart activity is not altered. Fleckenstein and coworkers [24] have described this condition as an "utilisation insufficiency": "The reserves of energy-rich phosphate can no longer be utilised for the contraction of the myocardium when the electromechanical coupling reaction is inhibited. The prototype of such a utilisation insufficiency resulting from disturbed electromechanical coupling is the myocardiac insufficiency caused by the withdrawal of calcium".

The same authors found isoprenaline most suitable for restarting the inhibited utilisation of phosphate. As can be seen from Fig. 4, isoprenaline also removes the severe insufficiency caused by sodium citrate intoxication: pulse rate and venous pressure adjust to a normal value while the arterial pressure rises. As the infusion of citrate is continued, the effect of isoprenaline diminishes, but can be repeated as and when desired. As expected, isoprenaline has no influence on the calcium ion concentration, which reaches a marginal value after decreasing by an average of 2.0 mg%.

The administration of complexed calcium has no effect on the calcium ion concentration and the circulation. On the other hand, the curve rises steeply after administration of an identical amount of dissociated calcium salts and the circulatory insufficiency is removed at the same time.

The blood potassium curve recorded simultaneously shows the typical course: a temporary rise is manifest after dosage with isoprenaline and calcium ions but not after administration of complexed calcium (Fig. 5).

Conclusions

Our test results prove the point made in medical literature, *i.e.* that the effect of sodium citrate depends on the amount infused per unit of time. 10 mg/min, *i.e.* about 3 mg/kg/min of sodium citrate administered i.v., causes no acute disturbance in healthy cats, which means this amount is tolerated. After the administration of 30 and 50 mg/min corresponding to about 10—15 mg/kg/min of sodium citrate given i.v., severe circulatory insufficiency occurs after 10 and 20 minutes respectively as a result of the decrease in the calcium ion concentration in the blood. The decrease in the calcium ion concentration can be determined by the method described by us.

Relative tests showed that these alterations become much sooner noticeable if the animals are weakened by a loss of blood amounting to 1/100 to 1/10 of their body weight. The tolerance limit in such animals may be expected to range below 3 mg/kg/min of sodium citrate. In human medicine blood containing citrate is infused primarily to balance out blood losses. Here, too, reduced tolerance must be expected. If a concentration of 1 mg/kg/min of sodium citrate administered i.v. is considered harmless to humans [14], similar conditions exist in humans and cats.

The circulatory alterations observed after sodium citrate intoxication fit well into the description by Fleckenstein and co-workers of a utilisation insufficiency caused by the withdrawal of calcium. Analogous to the investigations carried out by these authors, the insufficiency observed in our experiments could be overcome by the administration of isoprenaline and calcium ions but not by complexed calcium. We shall shortly report on the results of experiments in which the electromechanical decoupling after electric stimulation of the heart can be proved by means of a transvenously introduced pacemaker.

Summary

After the infusion of sodium citrate a decrease in the calcium ion concentration in the blood of cats oc-

curs, which is influenced by the dose administered and the time unit. The decrease in the calcium ion concentration is determined by continuous fluorometric measurement of the calcium and is continuously recorded. Even a slight deficiency in calcium ions causes typical alterations in the electrocardiogram and in the nerve/muscle preparation. A more severe deficiency brings about circulatory insufficiency described by Fleckenstein and co-workers. This utilisation insufficiency can be counteracted by administering N-isopropyl-noradrenaline-sulfate (isoprenaline; Aludrin®) and calcium ions but not by complexed calcium.

Cats weakened by loss of blood exhibit toxic symptoms earlier than healthy animals after a dose of sodium citrate.

Zusammenfassung

Kontinuierliche Bestimmung von Calcium und Kalium im Vollblut von Katzen nach Infusion von Natriumcitrat

In entsprechender Dosierung und in Abhängigkeit von der Zeit kommt es bei Katzen nach Infusion von Natrium-Citrat zu einer Verminderung der Calcium-Ionen-Konzentration im Blut, die mit einer kontinuierlichen fluorometrischen Bestimmung von Calcium erfaßt und fortlaufend registriert wird. Bereits ein geringer Mangel an Calcium-Ionen verursacht typische Veränderungen im EKG und am Nerv-Muskelpräparat. Ein stärkerer Mangel an Calcium-Ionen führt zu einer Kreislaufinsuffizienz. Hierbei dürfte es sich um eine von Fleckenstein u. Mitarb. beschriebene Utilisationsinsuffizienz handeln, die sich günstig durch Gabe von N-Isopropyl-noradrenalin-sulfat (Isoprenalin; Aludrin®) und Calcium-Ionen, nicht dagegen durch Gabe von komplexgebundenem Calcium beeinflussen läßt.

Durch vorausgegangenen Blutverlust geschädigte Katzen zeigen früher toxische Erscheinungen nach Gabe von Natrium-Citrat als gesunde Tiere.

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Resorption der Soluta im Nephron nach Applikation der Etacrynsäure beim Menschen

Von J. Stříbrná und I. Sotorník

Unter technischer Mitarbeit von Z. Bambasová, A. Harvánková, D. Pourová

Eine der Ursachen des signifikanten saluretischen Effektes des neuen Diureticums Etacrynsäure (2,3-Dichlor-4-(2-methylenbutyryl)-phenoxy-Essigsäure*) ist der breite Bereich ihrer Wirkung auf den transtubulären Transport des Natriums im Nephron. Aus einer Reihe experimenteller und klinischer Studien [2, 4, 7, 9, 10, 11, 13, 15, 16, 18, 21] ist bekannt, daß dieses Diureticum die Natrium-Rückresorption nicht nur im ascendenten Schenkel der Henle'schen Schleife, besonders auch im proximalen oder distalen Nephronabschnitt, herabsetzt.

Die saluretische Reaktion auf Verabreichung der Etacrynsäure erhöht sich in linearer Abhängigkeit von der sich steigernden Dosis von 50—250 [bzw. 400 mg] p.os [6, 14, 17, 24]. Die Wirkung des iv. verabreichten Na-Salzes der Etacrynsäure übersteigt auch hohe peroorale Dosen, was durch die Differenzen in der Konzentration dieses Diureticums im Blut und dadurch auch in der Niere in Abhängigkeit von der Applikationsweise erklärlich ist.

In dieser Untersuchung stellten wir uns die Frage, ob die Ursache der ansteigenden saluretischen Wirkung der Etacrynsäure in Abhängigkeit von der Dosis bzw. Applikationsweise die Verstärkung des Inhibitionseffektes auf den Na-Transport, vor allem an den Prädilektionsstellen im Nephron ist, oder ob auch die Lokalisation der Wirkung allmählich erweitert wird.

*) Edecrin®; in Deutschland: Hydromedin®, Hersteller Sharp & Dohme GmbH, München.

Von diesem Gesichtspunkt aus studierten wir Veränderungen der tubulären Na-Resorption nach peroraler und intravenöser Darreichung der Etacrynsäure an gesunde Personen bei erhaltener Wasser-Diurese.

Methodik

Wir verfolgten die Wirkung der Etacrynsäure bei Personen, die keine Störung des Wasser- oder Elektrolytstoffwechsels aufwiesen und deren renale Funktionen sowie der Blutdruck normal waren. Ihre Nahrung vor der Untersuchung enthielt einen normalen Protein- sowie Kochsalzgehalt, die Flüssigkeitszufuhr war frei und wurde am Tag vor der Untersuchung abends um 20 Uhr eingestellt.

Am Morgen des Untersuchungstages trank jede Person auf nüchternen Magen einen schwachen Hagebuttentee (Kaliumgehalt: 1,5—2,0 mäqv/l) in einer Menge von 20 ml/kg Körpergewicht, um die Diurese anzuregen. Der Urin wurde durch spontane Miktion gesammelt, anfangs in Intervallen von 30 min, später 10—15 min. Die Diurese wurde aufrecht erhalten, indem nach jeder Sammelperiode die untersuchte Person eine weitere Flüssigkeitsmenge erhielt, die identisch war mit dem Volumen des entleerten Urins plus soviel Milliliter, wie die Sammelperiode an Minuten betrug (zum Ersatz des Verlustes durch perspiratio insensibilis). Nach Stabilisierung der Diurese (d. h. wenn die Minutendiurese bei drei aufeinanderfolgenden Sammelperioden nicht um mehr als 1 ml/min differierte) wurde die Etacrynsäure appliziert und die Urinsammlung sowie die Erhaltung der Diurese nach peroraler Darreichung 90 min, nach iv. Applikation 45 min lang weiter fortgesetzt.

In der Gruppe von zehn Untersuchten (neun Männer und eine Frau im Alter von 17 bis 52 Jahren, Durchschnittsalter 39 Jahre), erhielt nach Stabilisierung der Diurese jede Person 150 mg Etacrynsäure peroral verabreicht, in einer weiteren Gruppe von acht Untersuchten (fünf Männer und drei Frauen im Alter von 21 bis 52 Jahren, Durchschnittsalter 36 Jahre), wurde jeder Person das Na-Salz der Etacrynsäure in einer Dosis von 50 mg iv. appliziert. Die Wirkung des Diureticums bewerteten wir durch Vergleich der Befunde aus der Stabilisierungsperiode der maximalen Diurese (Durchschnitt aus drei Sammelperioden) mit Befunden aus der Sammelperiode, in der nach

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THE STIMULATING INFLUENCE OF SODIUM CITRATE ON CELLULAR REGENERATION AND REPAIR IN THE KIDNEY INJURED BY URANIUM NITRATE[1]

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In 1916 MacNider (1) reported that sodium carbonate could be used to protect the dog's kidney against the toxic action of uranium nitrate. Later Goto (2) showed that sodium bicarbonate afforded the same type of protection against the substance. Whipple and his associates (3) found that hemoglobin gives protection for the kidney against injury by mercuric chloride. Recently Selye (4) made use of testosterone proprionate to successfully protect the kidney of the mouse against mercuric chloride poisoning, and Kety and Letonoff (5) have reported the use of sodium citrate as an effective agent in the treatment of lead poisoning. There is no apparent correlation between these various factors which offer protection against heavy metal injury.

In this paper evidence is presented to show that sodium citrate leads to recovery following a lethal dose of uranium nitrate by facilitating repair and regeneration in the injured renal epithelium.

METHODS. Forty-six female dogs between the ages of one and three years, with weights varying between 5 and 12 kgm. were used in the experiments. All of the animals were maintained on a regular kennel diet of cooked meat scraps and uncooked bones, supplemented occasionally by Purina dog chow. The intake of water was not restricted.

Each animal received a single subcutaneous injection of uranium nitrate, 5.0 mgm. per kgm. of body weight in 0.5 per cent aqueous solution (Merck, lot S-7197). Prior to receiving uranium nitrate certain of the animals were given daily, for five successive days, intravenous injections through the jugular vein of a saturated aqueous solution of tri sodium citrate 0.33 cc. (approximately 230 mgm.) per kgm. of body weight. On the fifth day the uranium nitrate was injected in the amount and manner as before described. The intravenous tri-sodium citrate injections were then continued for five additional successive days following the uranium. The amount and timing of the citrate injections is explained by the fact that these studies grew out of other studies in which sodium citrate was used as an anticoagulant (6).

Studies of the urine consisted of specific gravity determinations, qualitative tests for albumin, microscopic studies, and determination of phenolsulphonphthalein elimination. Studies of the blood were confined to determinations of non-protein nitrogen by the method of Folin and Wu (7) and of the carbon dioxide combining power of plasma by the method of Van Slyke and Cullen (8).

Anatomical studies were made on all animals which died, on certain animals which were sacrificed, and on survivors from which biopsy material was removed. Paraffin sections stained with hemotoxylin and eosin were made routinely from the kidney and liver.

[1] Aided by funds from the Josiah Macy, Jr. Foundation and the John and Mary R. Markle Foundation.

Results. In the first group of 13 control dogs which received one subcutaneous injection of 5 mgm. of uranium nitrate alone, all of the animals by the seventh day showed signs of a severe intoxication such as the refusal of food, nausea and vomiting, rapid loss of weight, inability to stand, and finally anuria. Between the ninth and thirteenth days 12 of the 13 animals died.

In the second group of 14 animals which received uranium nitrate preceded for 5 successive days and followed for 5 successive days by intravenous injections of 0.33 cc. per kgm. of a saturated solution of sodium citrate one animal died. The survivors in this group showed little, if any, clinical evidence of the intoxication. None of them failed to eat, and none of them showed any signs of nausea or weakness.

Albumin, red blood cells, and casts appeared in the urine of all animals within twenty four hours after injection of uranium. These changes persisted in the control group until an anuria developed, while in the "citrate" group the amount of albumin and the number of casts and red blood cells was diminished by the end of the second week and had largely disappeared by the end of the third week. Phenolsulphonphthalein elimination in both groups was reduced to virtually a negative reading and failed to increase in the control group. In the "citrate" group the output of the dye was increased to an average of 10% by the twelfth day and to 30% by the end of the third week.

The blood non-protein nitrogen in the control and "citrate" groups rose to comparable levels of retention during the first week. This level in the control group increased until death, while in the "citrate" group there was a definite decrease by the tenth or eleventh day. By the end of the third week the blood non-protein nitrogen in the animals of this group had returned to within normal limits.

The blood plasma carbon dioxide combining power showed about the same fall in the reserve alkali of both the control and "citrate" animals during the first week. By the ninth day, however, the "citrate" animals showed a rise toward normal which was completed by the end of the third week. In the control animals the diminished carbon dioxide combining power persisted until death.

Histological studies were made of the kidneys from a group of control and "citrate" animals sacrificed in pairs at intervals of 3, 6, 8, 10, and 12 days after the injection of uranium nitrate. In both groups, at the end of 3 days, the studies show the same apparent degree of injury with edema and early necrosis of the convoluted tubules especially of the proximal convolution. Figures 3 and 4 show comparable areas in the cortex of the kidneys of a control and of a "citrate" animal both three days after the commencement of the uranium intoxication.

In the 6 day animals histological studies show there is a more advanced injury with necrosis especially well seen in the proximal convolution of the tubule. Limited sloughing of the necrotic tissue has taken place and a flattened type of epithelium emphasized repeatedly by MacNider (9) is beginning to reline the necrotic tubules. At this stage these regenerative epithelial changes begin to be more prominent in the "citrate" animals. Figures 5 and 6 show comparable

areas in the cortex of the kidneys of a control animal and of a "citrate" animal both six days after uranium.

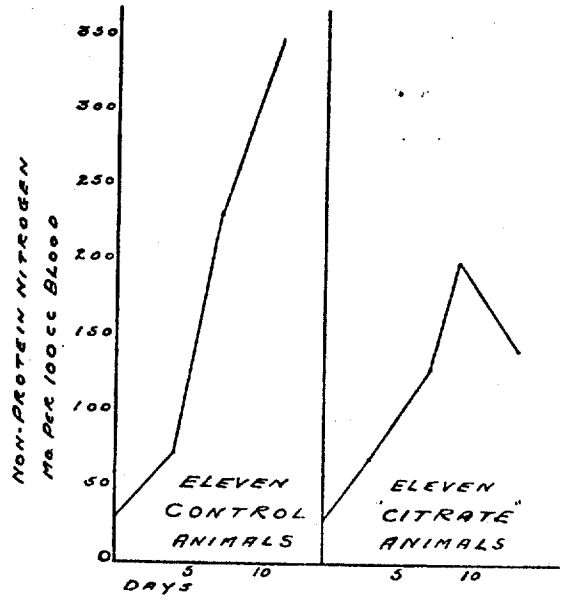

FIG. 1

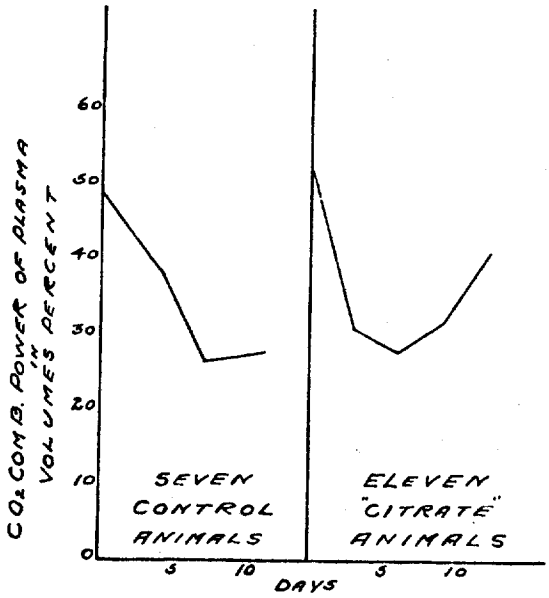

FIG. 2

The 12 day animals show by far the most striking difference seen in the histological studies. In the "citrate" animals approximately half of the tubules

FIG. 3. CONTROL DOG (K-22), 3 DAYS AFTER URANIUM NITRATE
Most of convoluted tubules are necrotic

FIG. 4. "CITRATE" PROTECTED DOG (K-47), 3 DAYS AFTER URANIUM NITRATE.
COMPARABLE NECROSIS OF CONVOLUTED TUBULES

are open and clear of necrotic material, and are relined by newly formed flattened epithelium. In the control animals there is evidence of a much smaller amount

FIG. 5. CONTROL DOG (K-21), 6 DAYS AFTER URANIUM NITRATE
Necrotic tubular epithelium is conspicuous

FIG. 6. "CITRATE" PROTECTED DOG (K-26), 6 DAYS AFTER URANIUM NITRATE.
COMPARABLE NECROSIS OF CONVOLUTED TUBULAR EPITHELIUM

of uncompleted regeneration. The tubules are still filled with necrotic material and only a few have been relined by flattened cells. Figures 7 and 8 are from

FIG. 7. CONTROL DOG (K-15), 12 DAYS AFTER URANIUM NITRATE
Only a rare tubule shows evidence of "healing"

FIG. 8. "CITRATE" PROTECTED DOG (K-23), 12 DAYS AFTER URANIUM NITRATE
One-half of the tubules are relined by newly formed flattened epithelium.

sections of the cortex of the kidneys of a control animal and of a "citrate" animal, both 12 days after the injection of uranium nitrate.

Discussion. The first striking observation in these studies is that 92% of the dogs receiving intravenous injections of sodium citrate survived the subcutaneous administration of 5 mgm. of uranium nitrate per kilogram of body weight, while the survival rate in the uranium dogs without citrate was 8%.

All observations—clinical, chemical and histological—indicate, by the usual methods of histological study and cytological analysis, a comparable degree of kidney injury in the "citrate" and in the control animals during the first few days following the injection of uranium. During the second week there is developing in the "citrate" kidney a marked amount of repair while in the control no comparable changes take place. By the twelfth day the repair process in the "citrate" kidney is nearing completion and the animals are well on the road to recovery, while the control animals lacking such reparative changes are usually dead before the twelfth day.

It would be difficult to isolate the factors involved in the repair process or to know the mechanism of their action and no attempt has been made to do so in the present study. The chief function of this paper is to report the unusual picture of cellular repair and regeneration produced by the action of sodium citrate on the kidney injured by uranium nitrate.

CONCLUSION

In the kidney injured by uranium nitrate the use of sodium citrate facilitates repair and regeneration of the injured tubular epithelium and results in the recovery of 92% of the animals receiving a lethal dose of uranium nitrate.

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by certain industrial concerns for periodically testing the urine of those exposed to radioactive compounds.

Even though these studies are incomplete a word or two about the immediate problem that attracted our interest to this method, viz., the protective influence of sodium citrate against uranium injury[2] might not be out of place. The 2 dogs which did not receive citrate (39-38 and 39-42) died on the 9th and 8th days respectively while the 2 dogs which did receive citrate injections (42-33 and 43-34) are both alive 12 months after the uranium injection. Both the rate of disappearance from the blood stream and the highest percentage recovery in the urine (88%) were in the dogs that received citrate (dogs 43-33 and 43-34). This might indicate that citrate brings about a more rapid elimination of the heavy metal, but if ⅔ of the heavy metal is eliminated in the first 24 hours without citrate it is difficult to see how citrate administered as late as 67 hours after uranium can be equally effective in protecting against the heavy metal (see next paper).

Summary. 1. A method is described whereby the alpha-particle radioactivity of uranium can be utilized to follow its course in the body. 2. Four hours after the intravenous injection of a minimal lethal dose of uranium nitrate (5.0 mg/kg) practically none of the injected heavy metal can be detected in the blood plasma. 3. Within the first 24 hours after the intravenous injection, approximately ⅔ of the uranium is eliminated in the urine. 4. Similar tracer studies have been applied to dogs protected by sodium citrate injections, but no satisfactory data have been obtained to explain the manner in which sodium citrate protects against a lethal dose of uranium nitrate.

14703

Studies on Heavy Metal Poisoning: II. Sodium Citrate Therapy Effective 67 Hours After Uranium Injury.*

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Last year we reported the survival in 13 of 14 dogs (92%) that had received intravenous injections of sodium citrate for 5 days before and 5 days after a lethal dose of uranium nitrate. Twelve of 13 control dogs (without citrate) succumbed to the same dose of heavy metal (5.0 mg/kg). One week after the injection of uranium the degree of azotemia, of acidosis, of alterations in the urine, and of histological change in the kidney in the two groups of dogs was similar.[1] Apparently something happened between the 5th and 8th day that determined the fate of the dogs.

The timing of the citrate injections in these experiments, *i.e.*, before and after the administration of uranium, was related to the fact that these studies grew out of other studies in which sodium citrate was used as an anticoagulant. Because of theoretical and practical considerations we have repeated these experiments but have given the citrate injections only *after* the uranium poisoning.

Methods. The methods were the same as those employed in the previous study[1] except that the daily intravenous citrate injections (0.33 cc/kg—approximately 0.23 g—of a saturated aqueous solution of tri-sodium citrate) were started at varying intervals *after* the subcutaneous injection of uranyl nitrate ($UO_2(NO_3)_2 \cdot 6H_2O$, 0.5 mg/kg in 0.5% aqueous solution). Blood samples for NPN and CO_2 determinations and urine samples

* This investigation was aided by a grant from the John and Mary R. Markle Foundation.

[1] Donnelly, G. L., and Holman, R. L., *J. Pharm. and Exp. Therap.*, 1942, 75, 11.

TABLE I.
Effect of Sodium Citrate Injections on Survival in Dogs Given a Lethal Dose of Uranium Nitrate.

Group	First citrate inj. given	Citrate inj. continued for days	No. of dogs	No. surviving	% survival
I	Simultaneously	10	5	5	100
II	(a) After 17 hr	10	5	5	100
	(b) " 17 "	5	6	5	83
III	" 67 "	5	4	4	100
	Total		20	19	95

for PSP, protein, and microscopic analyses were collected on the 3rd, 7th, and 12th day after the injection of the heavy metal.

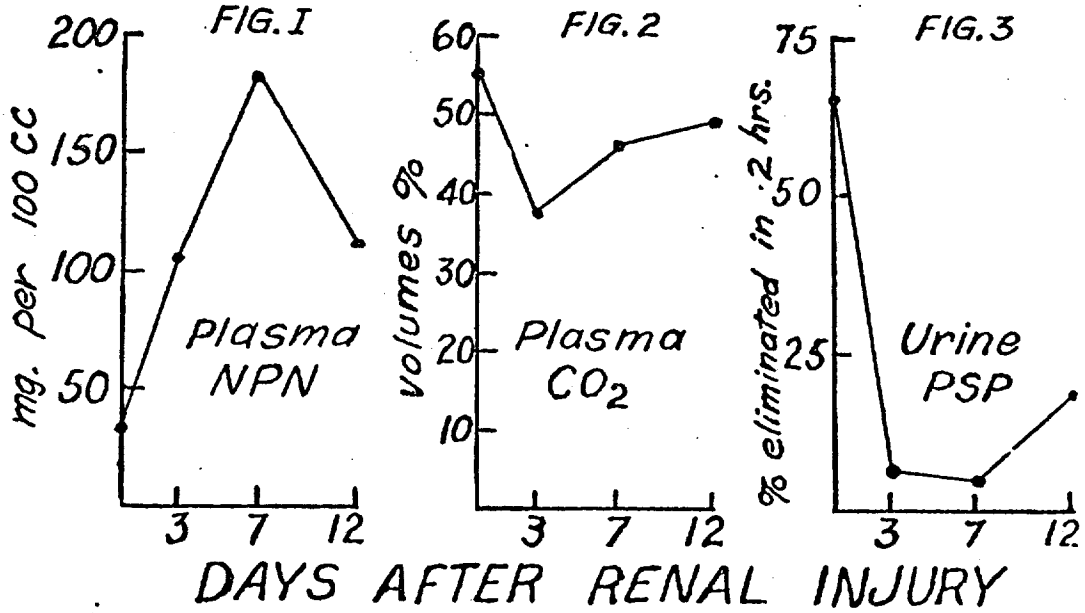
Experimental Observations. Table I gives the survival figures for the group of 20 dogs. Sodium citrate injections started as late as 67 hours after the administration of uranium and continued for only 5 days were as efficacious as 10 daily injections started immediately after the injection of the heavy metal. And the survival figure for the entire group, 95%, is the equivalent of that obtained in the previous series (with citrate injections before and after the uranium injections) in which 13 of 14 dogs (92%) survived.

In Fig. 1, 2, and 3 are presented the changes in NPN, CO_2, and PSP following the heavy metal injury. The points plotted represent the average for the entire group as obtained on the 3rd, 7th, and 12th days after the uranium injection and are practically identical with those obtained in the previous study except that the CO_2 combining power of the plasma did not fall quite as low and started returning toward normal sooner than in the previous study.

The clinical condition of the dogs remained good throughout. All reactions were averted by giving the citrate injections very slowly. No abnormalities in the clotting of blood were noted at any time. The single fatality (Group IIb) occurred on the 12th day.

Anatomical studies in this series of dogs were limited to biopsy and necropsy material

Effect of Sodium Citrate Injections on Blood Chemical and Urinary Excretion Studies in Dogs Given a Lethal Dose of Uranium Nitrate.



obtained from 3 weeks to 9 months after the renal injury. The kidneys of the dog that died on the 12th day showed considerable evidence of regeneration. By the 3rd week approximately ⅔ of the damaged tubules were lined by newly formed flattened epithelium. Anatomical material at later periods showed a progressive decrease in the amount of this "flattened epithelium," and most of the kidneys examined 9 months after the injection of uranium showed normal broad columnar epithelium lining the proximal convoluted tubules.

Discussion. The practical implications of these studies on acute uranium poisoning are obvious. We have not made any studies on chronic poisoning by uranium. The only report that we know of in which sodium citrate has been used to counteract heavy metal poisoning is that by Kety and Letonoff[2] in which sodium citrate gave beneficial results in human cases of lead poisoning. It is possible that some of the success usually attributed to plasma and indirect whole blood transfusions might be due to sodium citrate.

The theoretical considerations of these studies involve not only the method whereby sodium citrate protects against uranium ions but also the pharmacological action of the heavy metal itself.

There are several points that militate against the currently accepted view that sodium citrate exerts its protective action by preventing or combating the acidosis associated with uranium nitrate injury:

1. The alkali reserve as expressed by the CO_2 combining power of the plasma is not maintained by sodium citrate [Fig. 2 and (1, Fig. 2)].

2. The acidosis is the result of and not the cause of the renal injury.[3]

3. Sodium citrate does not prevent the curious coagulative necrosis of the proximal convoluted tubules of the kidney which classically follows uranium nitrate injury (1, Fig. 3-8).

4. If the results in the preceding paper in this series[4] are confirmed—namely, that approximately ⅔ of the uranium is eliminated in the urine in the first 24 hours—it is difficult to see how citrate administered 67 hours later can counteract the effects already produced by uranium.

Rather our data indicate that sodium citrate maintains one or more vital equilibria during the critical period (5th-8th day) when regeneration is taking place. This would indicate "replacement" or "supplemental" therapy which is directed at something more fundamental than the acid-base balance.

Possibly uranium damages a specific substance which is formed or concentrated in the cortex of the kidney. Indirect evidence pointing to a selective action by uranium is not lacking: (1) there is little or no reaction about the injection site in the subcutaneum, the obvious site of maximum concentration of uranium ions; (2) within 12 hours after subcutaneous injection there is functional evidence of renal injury (proteinuria) and at the end of 24 hours anatomical evidence of selective damage in the proximal convoluted tubules is demonstrable. Thus the lack of correlation between the concentration of uranium ions and the site of anatomic injury and between the time of maximal concentration at the site of damage (if ⅔ of the total amount injected is lost from the body in the first 24 hours) and the time of effective therapy (as late as 67 hours after the injection) could be used to support the selective affinity hypothesis.

This selective "toxic action" of uranium might logically occur by competition between uranium ions or electrons and those of another cation (perhaps a trace element) in an enzyme system with consequent inhibition of that system. The fact that uranium is hexa-valent makes this hypothesis attractive; and some of the other findings associated with uranium injury (glycosuria and increased susceptibility of pregnant animals[5]) could be put on a more logical basis if this or a similar hypothesis could be established.

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Whether or not this hypothesis holds true, we believe that our data on sodium citrate protection against uranium injury point to the maintenance of one or more vital equilibria (possibly the "citric acid cycle" of carbohydrate metabolism[6]) while the normal processes of repair are taking place, for we have no evidence that repair is accelerated or altered in any way.

Summary. Nineteen of 20 adult dogs survived a lethal dose of uranium nitrate (5.0 mg kg) when large doses of sodium citrate (0.23 g kg day for 5-10 days) were injected intravenously. The citrate injections were as effective when started as late as 67 hours after the heavy metal as when started immediately after the injection of uranium nitrate.

Objections are raised to the currently accepted view that maintenance of the alkali reserve is the mechanism whereby protection is afforded, and the suggestion is offered that sodium citrate supplies ions or molecules that keep the vital "citric acid cycle" of carbohydrate metabolism going while recovery takes place.

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Maternal Inheritance of Protoporphyrins of the Harderian Glands in Mice.

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Data indicating a parallelism between the amount of protoporphyrins in the Harderian glands of mice and intrinsic susceptibility to cancer of the mammary gland have been published.[1,2] Mice belonging to inbred strains with a high incidence of spontaneous mammary tumors have a very high concentration of protoporphyrins (deep red fluorescence under ultraviolet light); mice of strains with intermediate degrees of tumor susceptibility show an intermediate "pink" fluorescence; and mice of cancer-resistant strains exhibit very little or none of the fluorescence characteristic of protoporphyrins. Moreover, female mice of a cancer-susceptible strain (C_3H) have a higher concentration of protoporphyrins in their Harderian glands than males of the same strain and age.[3] *Only* female mice of a cancer-susceptible strain develop breast cancer spontaneously.

The problem of immediate interest is, can further studies on the determination of protoporphyrin concentration throw additional light (or reveal further parallelisms) on their level and the phenomena of susceptibility and resistance to spontaneous cancer? Among the most interesting (and perhaps the most important) observations in the analysis of the genetic influence on cancer susceptibility and resistance in mice is the finding that susceptibility to cancer of the breast in the F_1 individual is determined by the female parent (maternal inheritance).[4]

Chart I presents the data obtained on the red fluorescence (hence protoporphyrin concentration) in mice of the (a) C_3H strain (high or maximal protoporphyrin in the Harderian gland and high cancer susceptibility), (b) JK strain (minimal or low protoporphyrin and low cancer susceptibility), and (c) the 2 F_1 classes obtained by crossing mice of the parental stocks in both directions. The first F_1 generation was obtained by crossing a

* This experiment has been made possible by grants from The Anna Fuller Fund and The Jane Coffin Childs Memorial Fund for Medical Research.

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BIOCHEMICAL DETERMINANTS OF INFECTION*

The Ludwig Kast Lecture†

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GEORGE BERNARD SHAW affected great scorn for medical science and particularly for the germ theory of disease. "The characteristic microbe of a disease might be a symptom instead of a cause" he asserted in the preface of his play "The Doctor's Dilemma." Few are those who take seriously Shaw's views regarding science. Yet, I wonder at times whether his aphorism was not a prophetic statement—even though somewhat fanciful in wording—of a problem which has come to occupy a prominent place in the field of infectious diseases.

Consider for example the perplexing riddles posed by the existence of silent or latent infections. Pathogens of all kinds—protozoa, fungi, bacteria, rickettsia, viruses can persist in an active form in the tissues of a susceptible host, without causing detectable lesions or symptoms. These latent infections can remain unnoticed for the life time of the infected individual, the host and the parasite ignoring or at least tolerating each other under normal conditions. Let the proper kind of physiological disturbance occur in the host, however, and the equilibrium is upset. At times, probably, the parasite is eliminated altogether. More often, it starts proliferating in an uncontrolled manner, the latent infection evolving into overt disease. Shaw was wrong of course in stating that the microbe was the consequence of the disease. But there was truth in his aphorism since in many cases something has first to happen to the patient before the microbe can multiply extensively enough to cause symptoms and lesions.

The problem of latent infections acquires large practical importance from the fact that many infectious diseases in our communities are caused

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† In honor of Dr. Ludwig Kast who first suggested the Graduate Fortnight.

by microorganisms which are widely distributed among normal, healthy members of the population. There is little doubt that a great part of the increase in the prevalence of infectious diseases observed under certain disturbed social conditions is due to the fact that widespread physiological misery tends to activate latent endemic infections. A group of Danish physicians who were interned in German concentration camps during the last war have made observations which are very instructive in this respect.[1]

Among infectious diseases, it was not the exotic, unusual epidemics, such as typhus, cholera, or even bacillary dysentery, which proved the most troublesome in their experience, but rather ordinary skin ailments, colds, bronchopneumonias, staphylococcus infections, pulmonary tuberculosis – in other words, the type of disease, benign or severe, caused by microorganisms endemic in the normal European communities. Increase in contact infections could hardly account for the aggravation of these endemic diseases. Far more important certainly was the loss of natural resistance caused by malnutrition and other forms of physiological misery. It was remarkable indeed that most of the Danish internees overcame their microbial maladies shortly after their return to a normal environment and often without the help of specific therapy. Rapid recovery was the rule even in the case of tuberculosis, though no antimicrobial agent was then available for its treatment. The happenings in the concentration camps are directly relevant to our present topic because they can be regarded as the magnified manifestation of a state of affairs observed daily in medical practice.

During the past two decades, many types of substances, procedures, or accidents have been found capable of bringing about conditions under which latent microorganisms can manifest their potential pathogenicity and cause overt disease in man, animals, and plants. We shall briefly mention a few examples, selected not on the ground of their practical importance, but merely to illustrate two points: on the one hand, the variety of agents which are capable of *provoking* – as the expression goes – latent microorganisms into activity; on the other hand, the range of pathological states which can thus be evoked out of symptomless infections.

Intranasal injection of sterile broth or unheated serum into the nasal cavity of normal healthy mice brings about in a large percentage of these animals the development of pulmonary lesions caused by a variety of filtrable viruses latent in their lungs.[2-4]

In certain strains of mice the stresses and hormonal activity associated with reproduction and lactation determine the occurrence of mammary carcinoma in a large percentage of animals infected with the Bittner virus, whereas this infection remains symptomless in males or in virgin females.[5]

Mice or rats treated with large amounts of cortisone, or subjected to intense radiation, or placed on certain deficient diets, develop fatal pseudotuberculosis caused by Corynebacterium pseudotuberculosis murium.[6-10]

Cortisone, as is well known, can enhance many various types of latent infectious processes (reviewed in references 11 and 12), causing for example the activation of latent trypanosomiasis in a very large percentage of normal monkeys.[13]

Physical trauma brings about in dogs the proliferation of latent clostridia which convert traumatic shock into irreversible shock through the action of their toxins.[14]

In man the fever blisters which are caused by the herpes simplex virus and follow up many types of febrile episodes,[15] the moniliasis and aspergillosis associated with debilitating diseases or following treatment with antibacterial drugs,[16, 17] the reawakening of old tuberculous lesions by any form of physiological misery, the activation of toxoplasmosis by childbirth,[18] are but a few examples of latent infections which remain unnoticed under normal conditions but are provoked into activity by non-specific disturbances.

* * * *

While there is no knowledge of the precise mechanisms by which latent infections can be converted into active disease, at least two of the possibilities that first come to mind appear to be ruled out. The activation of the infectious process does not seem to result from the loss by the host of specific protective immunity—humoral or cellular; thus the herpetic individual who develops fever blisters possesses in his serum neutralizing antibodies against the herpes virus. Nor does the provoking agent exert its effect through increasing the virulence of the potential pathogen, as shown by the following example. Normal guinea pigs inoculated with BCG do not develop progressive infection although living bacilli persist for long periods of time in their tissues. If however the animals are made to inhale silica dust, BCG tuberculosis develops with production of cavities at the site of the silicotic reaction and death often ensues. Yet, the bacilli recovered from the lesions are found to be just as attenuated in virulence as those originally injected into the guinea pig. It is apparent therefore that silicosis acts as a provoking agent of the tuberculous process, not by increasing the virulence of the BCG bacilli, but by paralyzing the resistance which the tissues of the guinea pig normally possess against these microorganisms.[19]

Whatever the nature of the reactions through which provoking agents decrease the resistance of the host, either systemically or locally, one of their effects in all likelihood is to alter the physicochemical environment in the tissues involved. This in turn is bound to influence the severity of the disease since the multiplication and viability of microorganisms, as well as their toxic properties, are conditioned by the composition of the environment. In order to understand the role played in the evolution of the infectious process by changes in the physicochemical characteristics of the tissues, it would be necessary to have a precise knowledge of the conditions under which microorganisms find themselves in vivo. Unfortunately, very little is known in this regard.

At first sight, it would appear that the in vivo environment is merely the physiological *milieu intérieur* determined by the homeostatic mechanisms of the body. In reality, however, the particular environment in which the infectious process follows its course is far different from that termed "physiological." Most microorganisms are taken up by phagocytic cells as soon as they are introduced into the body, the first phase of infection being thus intracellular. Only in a later phase does necrosis cause them to be released into the abnormal extracellular environment of the lesions. The problem is hardly simpler for extracellular infections. It is true that the microorganisms find at first in blood and lymph conditions corresponding to those of the physiological *milieu intérieur*. But the host soon responds to the presence of parasites by the complex and variable reactions referred to under the generic name of inflammation. There is no doubt that the inflammatory zone differs biochemically from the normal tissues and body fluids. Although little is known of the physicochemical conditions prevailing both intracellularly within phagocytic cells and extracellularly within the lesions, a few facts appear worth recording.

It has been demonstrated that leukocytes can produce large amounts of lactic acid and it is probable that their intracellular pH following phagocytosis is rather low (reviewed in reference 20). Similarly, it is certain that the pH of inflammatory exudates is commonly very acidic (values as low as pH 5.3 have been reported) in part at least as a result of the local accumulation of lactic acid produced by inflammatory cells and perhaps also by fixed tissue cells[21, 22] (reviewed in reference 20).

On the other hand it is very likely that the oxygen supply to many lesions or parts of lesions is greatly reduced by the crowding of migra-

tory cells, vascular thrombosis, and other interference with blood flow. These same factors contribute also to increasing locally the CO_2 tension.

Finally, all lesions being the sites of necrotic changes, a great variety of substances resulting from the breakdown of tissue cells accumulate in them; many of these substances are known to affect profoundly the fate and behavior of pathogenic agents (reviewed in reference 20).

Thus it is clear that microorganisms exist in vivo in an environment which is highly complex and abnormal. In final analysis, the physicochemical characteristics of this environment are determined by the biochemical activities of tissue cells; these activities are not constant but instead vary with the metabolic state of the individual. Since the range of metabolic variations is certainly very large, and knowledge concerning this subject very scant, there is much artificiality in the selection of any particular item for emphasis. Granted the arbitrary character of the choice made in the following discussion, it seems worth pointing out nevertheless that certain physiological disturbances are probably reflected in the metabolism of inflammatory cells, modifying their phagocytic activity, their ability to produce lactic acid, even resulting in the abnormal production of other organic acids. As examples, one need only mention the ketosis of uncontrolled diabetes or starvation, or the low glycolytic activity exhibited by the leukocytes of the diabetic individual,[23] or the altered metabolism of tissue cells to which cortisone has been added in vitro.[24]

Before discussing the possible relevance of metabolic disturbances to the phenomena of infection, it may be useful to mention that the various organic acids produced by tissue metabolism differ profoundly in their ability to support bacterial growth. Experiments in vitro with tubercle bacilli, staphylococci and Friedländer bacilli have revealed that lactic acid in concentrations of the order of 0.01 M can inhibit the growth of these organisms and moreover can bring about their death if the environment is anaerobic. The bacteriostatic and bactericidal effects are the more pronounced, the more acid the reaction. Thus, lactic acid possesses antibacterial activity precisely under the conditions which normally exist in inflammatory areas. In contrast, most keto acids and polycarboxylic acids favor bacterial growth under the very same conditions, and indeed can protect microorganisms against the toxic action of lactic acid.[20, 25]

Needless to say, it is not easy to prove that these in vitro findings

have any bearing on the events of infection in vivo. It seems reasonable to believe nevertheless that general metabolic disturbances are reflected in the biochemical activity of phagocytic and inflammatory cells, and must thereby affect the course of infectious processes through their action on the fate and activities of microorganisms in the tissues. Suggestive evidence of this relationship is found in the great susceptibility to infection associated with uncontrolled diabetes and with acute starvation. It is of interest in this respect that the resistance of starved and diabetic individuals becomes normal again when these patients are returned to a satisfactory nutritional state, or when their diabetes is brought under control by adequate insulin therapy. In other words, it appears that susceptibility to infection in these cases is the expression of a reversible disturbance, probably biochemical in nature.

Knowledge is not sufficient to permit a systematic analysis of the mechanisms through which metabolic disorders of the host can affect susceptibility to infectious diseases. Indeed, it is only during recent years that attempts—still very primitive—have been made to study this problem experimentally. We shall outline briefly some of the results obtained in trying to modify the course and outcome of infection by treatments designed to alter the metabolism of the infected animals.

* * * *

There is a general belief that malnutrition plays an important part in increasing susceptibility to infectious diseases. Surprisingly enough, however, it is not easy to find either clinical or experimental evidence to support this view. As has been aptly said the relationship is "made conspicuous more by reiteration than by demonstration."[26] In fact, there is on record well documented evidence that experimental animals fed diets deficient in some essential growth factor can be more resistant to infection than animals fed complete diets (reviewed in references 26 and 27). Instances to the case are thiamine deficiency in experimental poliomyelitis of mice and PABA deficiency in malaria of rats and monkeys.[28] The complexities of the relation between general nutritional state and susceptibility to infection have been critically analyzed in a number of recent reviews. [26, 27, 29-32]

Out of the mass of often conflicting data bearing on the relation between nutritional state and susceptibility, one general conclusion at least is beginning to emerge. The nutritional factors which have been

shown to be essential for growth and reproduction rarely if ever play a decisive and specific role in resistance to infection. True enough, aggravation of disease has been observed in infected animals fed diets deficient in one or the other vitamin. However, the results have been clear cut only in cases where the vitamin deficiency was so profound as to impair greatly the overall physiological well being of the animals.

Convincing evidence that the factors important for growth may differ from those which play a part in resistance to infection has come from the study of mouse salmonellosis. Resistance to this disease can be predictably increased by adding to the diet small amounts of a fraction from germinating wheat or barley (unidentified chemically) which does not modify the growth rate of uninfected animals.[33] Furthermore, certain types of diets which are very unsatisfactory from the point of view of their ability to support growth and reproduction in uninfected mice, do not in any way decrease their resistance to tuberculosis and may even be superior in this regard to diets that appear optimal according to ordinary nutritional criteria.[25, 30, 34] It is worth noting that despite common belief, the protein content of the diet does not have a direct bearing on resistance to infectious disease, at least as found in experimental infection with salmonella and tubercle bacilli.[27, 30, 32, 35, 36]

Recent findings indicate that in many cases at least the nutritional regimen alters resistance to infection indirectly through its effect on the metabolism of the infected animals. Mice fed ad lib diets considered optimal according to all nutritional criteria proved extremely susceptible to tubercle bacilli, staphylococci and Friedländer bacilli if deprived of food for periods of thirty hours (Tables I and II).[37, 38] Resistance to infection returned to normal within a few hours after food had again been provided to the animals.[38] Mice thus rendered susceptible by fasting lost little weight and regained it rapidly. In contrast, the course of infection was not aggravated in mice which were prevented from gaining weight by being maintained in a state of chronic undernutrition by limiting to a low, but constant level, either their total food intake, or the protein content of their diet[36, 37] (reviewed in reference 27).

It is difficult to attribute the infection-enhancing effect of acute but short periods of fasting to deficiencies in essential nutritional factors. It seems more likely that this effect is due instead to the transient production or accumulation in the tissues of the fasted animal of metabolites which favor the activities of microorganisms. In fact, it has been found

TABLE I.—EFFECT OF FASTING VS. CHRONIC UNDERNUTRITION ON MOUSE TUBERCULOSIS

Sherman Diet	*Weight Gain‡ (grams)*	*Cumulative number of mice (out of 10) dead at* 4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	9 weeks	10 weeks	11 weeks
ad lib	12.5	0	1	1	2	3	3	4	4
1 period of fasting*	10.5	1	3	3	4	4	5	7	7
2 periods of fasting**	7	2	4	4	5	6	7	9	10
3.5 gm. daily†	1	0	1	1	1	1	3	3	5

* Food withdrawn once for 30 hours 5 days after infection; otherwise diet given ad lib.
** Food withdrawn twice for 30 hours 5 days and 12 days after infection; otherwise diet given ad lib.
† This corresponds to two-thirds of normal food intake.
‡ Average (in grams) per uninfected, control mouse over a period of 6 weeks.

TABLE II.—EFFECT OF FASTING ON SUSCEPTIBILITY OF MICE TO INFECTION WITH STAPHYLOCOCCUS AUREUS OR KLEBSIELLA PNEUMONIAE

Infection	*Diet*	*Drinking Fluid†*	*Deaths Per Cent*
Staph 0.1 ml i.v.	ad lib	water	5
" " " "	Fasting*	water	75
" " " "	"	citrate	75
" " " "	"	lactate	35
Klebs. 10^{-3} ml i.v.	ad lib	water	0
" " " "	Fasting*	water	75
" " " "	"	citrate	100
" 10^{-4} " "	"	water	60

* Animals fasted for 24 hours before, and 12 hours after infection; food (pellets) otherwise supplied ad lib.
† Supplied throughout period of fasting and until end of experiment.

TABLE III.—EFFECT ON MOUSE TUBERCULOSIS OF CITRATE, GLUTARATE, LACTATE, AND ALCOHOL

Pellets ad lib Drinking fluid	*Cumulative number of mice (out of 10) dead at* 3 weeks	4 weeks	5 weeks	6 weeks
0.8% NaCl	0	1	5	10
2% Na citrate (pH6.0)	2	7	9	10
1% Na glutarate (pH6.0)	1	7	9	10
2% Na lactate (pH6.0)	0	0	3	7
5% alcohol in saline	0	2	7	9

TABLE IV—EFFECT OF DINITROPHENOL AND THYROID EXTRACT ON MOUSE TUBERCULOSIS

Pellets ad lib Drinking fluid containing (per cent) Dinitrophenol	Thyroid Extract	Cumulative number of mice* dead at 4 weeks	5 weeks	6 weeks
0	0	1	5	10
0.0025	0	2	4	6
0.005	0	4	7	10
0.01	0	4	7	10
0	0.003	2	3	7
0	0.01	4	8	10
0	0.03	10		

* Out of 10 mice.

that the death of tuberculous mice can be accelerated in a striking and consistent manner by adding to otherwise satisfactory diets certain metabolites such as sodium butyrate, citrate or glutarate; alcohol or lactate have no similar infection-enhancing effect, even when added to the diet in higher concentration[37] (Table III). It is of interest that mice can also be rendered more susceptible to infection with Salmonella typhimurium by injecting them at short intervals of time with solutions of sodium citrate or other metabolites.[39]

Like malnutrition, endocrine disturbances have long been implicated in susceptibility to infectious diseases. The infection-enhancing effect—in man and animals—of treatment with large amounts of cortisone has been described so often as to need no further mention (reviewed in references 11 and 12). Evidence has also been published that estrogen activates tuberculosis in rabbits and guinea pigs,[40] and thyroxine in mice.[41] Extensive experimentation in our laboratory has revealed that mice receiving daily in their diet amounts of thyroxine or of dinitrophenol sufficient to retard their normal weight gain exhibit an unusually high susceptibility to infection with tubercle bacilli and staphylococci (Table IV). The infection-enhancing effect of these metabolic stimulants is not immediate; it requires at least several days before becoming manifest.[37, 38]

The findings in experimental animals leave no doubt that one can at will decrease resistance to infection by simple manipulations of the dietary regimen or by causing certain metabolic or humoral disturbances; but though these effects are clear, their mechanism is very obscure. Acceleration of the death rate of infected animals could be the result of several independent causes which can be classified under at least three headings: a) The infection-enhancing substance or procedure might bring about in vivo an environment more favorable to the multiplication or survival of the infectious agent either by causing a depletion of the tissues in some growth inhibitor, or an increase in some growth accelerator. b) It might create conditions which change the infectious agent qualitatively, allowing it for example to express a wider range of its toxic potentialities. c) It might render the infected animal more susceptible to the effect of these toxins.

Before mentioning some of the specific reactions which one can assume to play a part in the enhancement of infection, it is important to emphasize that any disturbance, nutritional or metabolic, sets into motion a large number of complex processes with a multiplicity of ill defined secondary effects. These, often far removed from the initial disturbance, certainly involve in many cases various components of the endocrine system. Many of the observed cases of aggravation of disease might be due for example to an overproduction of cortisone. Hormones in their turn can indirectly bring about profound changes in the physicochemical characteristics of the in vivo environment. Thus cortisone, by reducing the inflammatory reaction, is likely to decrease the production of lactic acid at the site of the lesions. Or again hormones, through their control over the various inhibitors and activators of tissue proteases,[42] probably affect the stability of fibrin and collagen structures and thus modify the rate of exchanges between the lesion and the general body fluids (reviewed in reference 20). One might postulate also an effect of physiological disturbances on the activity and concentration in serum of the recently discovered properdin, a globulin which appears capable of inactivating some viruses and gram negative bacilli.[43] These few examples suffice to illustrate the complexity of the secondary effects through which physiological disturbances might affect the infectious process.

Among the responses of the total organism to the procedures capable of increasing susceptibility to infection there are some metabolic phen-

omena which may have a direct bearing on the behavior and fate of microorganisms in vivo. It has long been known for example that one of the first and most dramatic results of starvation is the utilization of the glycogen reserves of the body. Treatment with thyroxine or with dinitrophenol also brings about depletion of the glycogen stores, probably by interfering with the phosphorylation reactions essential to the synthesis of this polysaccharide.[44, 45] There is evidence on the other hand that phagocytic and other tissue cells depend upon glycogen for their metabolic activity, and probably derive their energy through the production of lactic acid by glycolysis.[46, 47] Thus it is conceivable that acute starvation, as well as administration of thyroxine or dinitrophenol, may activate infection indirectly through an influence on phagocytosis and on the accumulation of lactic acid in inflammatory areas.

Starvation has of course many other metabolic effects. Like uncontrolled diabetes, it brings about overproduction or accumulation of acetone bodies, particularly in its early phase. Since, as already mentioned, a number of keto acids can stimulate bacterial growth, and furthermore counteract the antibacterial activity of lactic acid, it seems not impossible that the increased susceptibility to infection often associated with diabetes and starvation is due in part to the ketosis brought about by these metabolic states.[20, 25, 37] The more severe course of salmonellosis in animals treated parenterally with citric acid[39] and of tuberculosis in mice receiving either citrate or glutarate in their diet[37] (Table III), can be cited to illustrate the profound effect exerted on infection by presenting the tissues with abnormal amounts of certain metabolites.

* * * *

Although the precise mechanism through which nutritional, metabolic, and hormonal disturbances affect the infectious process can be discussed only in terms of unproven hypotheses, some biological consequences of the phenomenon appear in a clearer light. We have already mentioned that withdrawal of food for only twenty-four hours can shorten in a dramatic manner the survival of mice infected with tubercle bacilli, staphylococci or Friedländer bacilli[37] (Tables I and II). Very recent observations have revealed furthermore that this effect obtains even in animals which have been vaccinated (with BCG in the case of tuberculosis, with the specific polysaccharide antigen in the case of Fried-

TABLE V.—COMPARATIVE EFFECTS OF VARIOUS DIETARY REGIMENS ON MOUSE TUBERCULOSIS AND ON WEIGHTS OF UNINFECTED ANIMALS

Percentage composition of diets					Weight gain† (grams)	Cumulative number of mice* dead at		
Skim milk	Wheat Flour	Cerelose	Peanut Oil	Citrate		3 weeks	5 weeks	7 weeks
4	45	30	20	—	3.8	0	1	2
4	45	50	—	—	3.0	1	7	10
4	45	40	—	10	0	3	8	10
34	65	—	—	—	8.8	0	0	4

* Out of 10
† Average per mouse over a period of 3 weeks.

länder infection). Thus, it is plain that a metabolic disturbance even very transient, can greatly increase receptivity to infection, a fact which may have far reaching epidemiological significance. Indeed, certain unsolved problems of epidemiology could be viewed in a new light if it were a general truth that the manner in which a given individual can deal with infectious agents varies widely from day to day independently of his immunological state and merely as a result of physiological stresses. The belief that certain diseases are more likely to be contracted if there is prolonged exposure to a source of the causative microorganism—for example in leprosy—might signify that the body is receptive to this agent only under certain transient physiological states. Contact and receptivity may be rarely coincidental.

It is likely, as we have emphasized, that the increase in susceptibility caused by the temporary deprivation of food is not the result of nutritional deficiencies as ordinarily thought of, but rather the consequence of the abnormal utilization or accumulation of some metabolite. If correct, this conclusion makes it doubtful that the criteria commonly used for the study of nutritional factors can serve to evaluate dietary regimens with regard to resistance to infectious diseases. The results presented in Table V illustrate for example that one cannot predict the effect of a given dietary regimen on susceptibility to tuberculous infection from its effect on the weight curve of uninfected animals. Many other facts point to the conclusion that the nutritional factors essential for growth and reproduction are not necessarily the ones most

important in determining receptivity to infectious agents and resistance to the diseases that they cause. These two latter properties are concerned, not with what is good for the host considered as an isolated system, but rather with the factors which control the interrelationships between host and parasites. It is even possible that receptivity to infection, and resistance to disease, are two different characteristics and are controlled by independent mechanisms.

In summary the metabolic determinants of resistance to infectious agents almost certainly differ from, and may even be independent of, those which control normal physiological behavior. Their identification demands the development of new experimental techniques, based on consideration of the factors which determine the multiplication and survival of microbial agents in vivo, the production of toxic substances, and the host response to the microorganisms and their products.

Ever since the formulation of the germ theory, the emphasis in the control of infectious diseases has been placed on a direct attack on the microbial parasites—by means of drugs, sera, vaccines, or public health measures. A new phase of the germ theory may well unfold itself if it becomes possible to identify the metabolic factors which permit so many individuals to harbor without manifestations of disease, microorganisms endowed with pathogenic potentialities. On the basis of this knowledge, it might some day become possible to design techniques of metabolic control that would help man live at peace with microbial parasites. Many of these, after all, constitute an inescapable part of his biological environment.

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The Nucleic Acid Content of Adrenal Tissue

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In the preceding paper by Professor Symington and Dr. Currie histochemical evide has been produced to indicate that a rise in the content of ribonucleic acid in the cytopl of the adrenal gland follows the administration of ACTH to human subjects and that suc rise is also evident in conditions of stress. We have attempted to confirm these conclusi by carrying out chemical analyses of the adrenal tissue used for histological examinati Ribonucleic acid phosphorus (RNA-P) and deoxy-ribonucleic acid phosphorus (DNA were estimated by the method of Schmidt and Thannhauser (1945) and lipid phosph (LP) was also estimated simultaneously. The results show that in normal tissue obtai either at operation or post mortem the RNA-P concentration lay between 15 and 28 mg. 100 grams tissue but that after the administration of ACTH values between 42 and 55 per 100 grams were obtained. A similar rise was found in LP but the DNA-P concentra remained unchanged at between 7 and 20 mg. per 100 grams. The result of administra of ACTH, therefore, was a very marked rise in ratio of RNA-P to DNA-P.

Although the figures for RNA-P in conditions of stress showed a wide scatter betw 15 and 44 mg. per 100 grams there was a definite correlation between the extent of the in RNA-P and the lipid pattern of the gland as expressed histologically by Professor Syming and Dr. Currie. Again the DNA-P was unchanged.

A similar effect was obtained in calves which had received 600 mg. ACTH over a peri of five days. Again the RNA-P showed a sharp rise in concentration and in absol amount per gland while the DNA-P showed a slight fall in concentration although t absolute amount per gland was almost unaltered.

The interpretation of these results is obscure but it is important to note that ribonuc acid is commonly considered to be associated with the process of protein synthesis references *see* Chargaff and Davidson, 1955). In spite of the obvious correlation in tim and place between the amount of ribonucleic acid in the cell cytoplasm and the amount protein being synthesized by a cell, the exact mechanism by which ribonucleic acid is sup posed to mediate in the process of protein synthesis is unknown and its association wi the synthesis of protein may indeed be purely coincidental.

From the results under review it is obvious that ACTH stimulates the formation of substance without at the same time increasing the amount of nuclear material, and it mi well be that although ACTH stimulates the formation of cellular protein, this protein cann normally exist in the cell cytoplasm except in association with ribonucleic acid whic therefore deposited as a structural unit along with the protein. But it is also possible t as Hechter (1953) has suggested, corticosteroidogenesis requires protein, the synth of which is controlled by ribonucleic acid produced in the gland under the influence of AC

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Professor F. T. G. Prunty:

The previous speakers have given a very clear and succinct exposition of many cu problems in the investigation of the adrenal. The remarks made by Dr. Dixon o relative stability of ACTH given by various routes were very important. For inst a valuable clinical use of ACTH was to determine the responsiveness of the ad cortex. In fully developed Addison's disease it was unresponsive, but in hypopituit various degrees of responsiveness were to be expected, depending on the degree of ad atrophy. An example was shown of data from such a patient who responded poor both intramuscularly and intravenously administered ACTH. Clinicians were also searc for patients who had adrenal cortical insufficiency on account of selective endoge ACTH lack, and the stability of the ACTH employed was of great importance in asse such a state. The patient just mentioned had also received injections of Professor You growth hormone together with ACTH without any perceptible enhancement of the resp This may have been due to having to limit the experiment to five days, but it was hoped some synergism of the two hormones might be demonstrated, especially as there w significant rise of the blood-sugar levels.

Dr. Bush's paper dealing with the metabolism of adrenal steroids indicated the along which the clinical chemist would have to be prepared to work. For specific purp it was now becoming necessary to be able to estimate corticoid or hydrocortisone metabol

h as the tetrahydro-compounds; 11 oxy-17-ketosteroids with differentiation of the 5α β isomers; dehydroepiandrosterone; pregnenetriol; aldosterone and its derivatives. thods were only available to achieve part of those objectives and the biochemical com- ity was considerable. The method of Norymberski was proving valuable for the estima- of some corticoid metabolites, as evidenced by the data presented on the patient with opituitarism and as shown by results obtained in the study of scurvy (Prunty, F. T. G., ton, B. E., McSwiney, R. R., and Mills, I. H., 1954, Ciba Foundation Colloquia on locrinology, London; 8, 324). This study had shown the value of histological examina- of the lipid of the fasciculata as pointed out by Dr. Currie and Professor Symington. uinea-pigs in which there was an increase of adrenal secretion as a result of acute scurvy as possible to find almost complete discharge of the lipid staining. With regard to nal cortical activity it had been noticed that in sections of the fasciculata from patients Cushing's syndrome some areas in sections stained by hæmatoxylin and eosin showed mns of cells with little lipid and dark-staining cytoplasm. These were probably etory cells and between them could be seen sinusoids considerably distended with blood These were recorded as being particularly helpful in assessing the increased activity e gland.

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Metabolic Disturbances and Infection

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r has been shown elsewhere that the life expectancy of tuberculous mice can be con- stently shortened by subjecting these animals to treatments which disturb their metabolism (Dubos, 1955*a*, *b*). More recent unpublished findings have revealed that the same treat- ents increase also the morbidity and mortality in mice infected with *Staphylococcus aureus*, *Klebsiella pneumoniæ*, *Salmonella enteritidis* and other bacterial species. The following procedures have been found to decrease consistently the survival time of infected animals:

(*a*) Feeding thyroxine or dinitrophenol in amounts sufficient to retard the normal weight ain of uninfected controls. The infection-enhancing effect of these two metabolic stimu- ants is not immediate; it requires at least several days before becoming manifest.

(*b*) Adding 1% sodium citrate or glutarate (but not lactate) to the diet. The infection- hancing effect of citrate or glutarate is particularly striking in diets deficient in fats and rotein.

(*c*) Fasting the animals for thirty-six to forty-eight hours immediately before or after fection. This effect is reversible, the animals recovering their normal state of resistance in forty-eight hours after having been returned to a complete diet *ad lib.* In contrast, course of infection is not aggravated in mice prevented from gaining weight by limiting low, but constant, level, either their total food intake, or the protein content of their (Table I).

e I.—Effect of Fasting Versus Chronic Undernutrition on Mouse Tuberculosis and on Weights of Uninfected Animals

erman Diet	Weight gain*	Cumulative deaths† at weeks							
		4	5	6	7	8	9	10	11
ad lib.	12·5	0	1	1	2	3	3	4	4
grams daily‡	1	0	1	1	1	1	3	3	5
asting once§	10·5	1	3	3	4	4	5	7	7
asting twice§	7	2	4	4	5	6	7	9	10

* Average (in grams) per uninfected mouse over a period of six weeks.
† Out of 10 mice.
‡ This corresponds to two-thirds of normal food intake.
§ Food withdrawn for thirty hours once (five days after infection), or twice (five days and twelve days after infection). Diet given otherwise *ad lib.*

the first phase of this study, the only criterion used to determine the effects of dietary metabolic factors on susceptibility to infection was the survival time of infected animals. eriments have since been conducted to determine whether the animals on the infection- ncing regimens die more rapidly than the controls because their tissues provide an *ivo* environment more favourable for bacterial growth, or because they are more eptible to the toxins produced by the bacteria.

order to study the fate of the microbial population *in vivo*, known numbers of micro-

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organisms were injected intravenously into mice and the animals were sacrificed at interva of time. The numbers of living organisms present in the blood, spleen, kidney, liver an lungs were then determined by culturing graded dilutions of blood or of organ homogenat on appropriate culture media.

In mice infected with virulent tubercle bacilli or B.C.G. and subjected later to nutrition or metabolic disturbances, an increase in the number of mycobacteria over that recover from normal animals can be detected within four to seven days after initiation of the di turbance. In reality, however, the length of this latent period does not provide an adequa picture of the dynamics of the phenomenon. It is an expression of the slow rate of mult plication of tubercle bacilli rather than of the character of the tissue response. This c be shown by using as infectious agents bacteria potentially capable of multiplying rapid *in vivo*, for example *Kl. pneumoniæ* or *Staph. aureus*.

Table II presents the results of an experiment in which mice infected intravenously wi

TABLE II.—EFFECT OF FASTING ON FATE OF *Kl. pneumoniæ* IN MICE

	Numbers† of bacteria recovered twelve hours after infection		
	Spleen	Liver	Blood
Normal mice	0, 0, 18	1, 1, ?	0, 0, 0
Fasted mice*	12, 390, 5,000	12, 500, 50,000	2, 23, 90

* Receive 5% glucose solution as drinking fluid.
† Numbers to be multiplied by 1,000 to give total value per organ.
Figures correspond to each of 3 mice per group.

$0{\cdot}2 \times 10^{-5}$ ml. of a culture of *Kl. pneumoniæ* (group C) were sacrificed twelve hours afte infection in order to determine the numbers of bacteria present in their organs. Some of the animals received no food during the forty-eight hours that preceded infection excep for the fact that their drinking fluid consisted of 5% glucose in saline. The controls were fed continuously a complete diet (pellets) *ad lib.* It can be seen that an incubation period as short as twelve hours was sufficient to allow the multiplication of immense numbers of bacteria in the various organs and in the blood of the fasted mice. In contrast, infection had not progressed in the control animals at that time.

Other aspects of the comparative dynamics of the infectious process in normal and fasted animals are revealed by a study of the results presented in Table III. In this experi

TABLE III.—EFFECT OF FASTING ON STAPHYLOCOCCUS INFECTION IN MICE

Treatment of mice	Deaths (out of 10)	Staphylococci† in liver at indicated times after infection		
		1 day	2 days	3 days
Fed pellets *ad lib.*	0	350	60	12
Fasted* +saline	5	440	510	160
,, +glucose	10	1,630	?	?
,, +sodium lactate	2	?	240	?

* Animals were fasted for 2 days before infection, and were given either saline, or 2% sodium lactate, or 5% glucose in saline as drinking fluid.
† Numbers to be multiplied by 1,000.

ment 0·1 ml. of a coagulase-positive culture of *Staph. aureus* was injected by the intravenou route, and the animals were sacrificed at various intervals of time after infection. It is see that the cocci disappeared progressively from the liver (and spleen) of the animals fe continuously a normal diet *ad lib.*, whereas they persisted and eventually gave rise to a overwhelming infection in the animals that were fasted or fed an incomplete die Administration of glucose in the drinking fluid aggravated the effect of fasting.

The results presented in Tables II and III are typical of those obtained in many simila experiments and suggest that the low resistance to infection exhibited by the animals o inadequate regimens is due in part to a failure of the bactericidal power of their organ A similar mechanism appears to account for the enhancement of staphylococcus infectio in mice caused by treatment with thyroxine (Table IV). Two facts, however, suggest tha

TABLE IV.—EFFECT OF THYROXINE ON FATE OF STAPHYLOCOCCI IN MICE

Thyroxine	Organ	Staphylococci* at indicated times after infection			
		1 day	2 days	3 days	4 days
Yes	Spleen	263	145	259	Death of mice
,,	Liver	1,503	750	450	
No	Spleen	540	7	3	1
,,	Liver	3,197	34	80	15

* Numbers to be multiplied by 1,000.

nterference with the clearing power of the organs is not the only mechanism through which metabolic disturbances can decrease resistance to infection. On the one hand there is no vidence that mouse tissues possess any bactericidal power for virulent tubercle bacilli, as hown by the fact that progressive infection can be established in normal mice by injecting hem intravenously with extremely small doses of these organisms (10^{-7} ml. of culture). et tuberculous infection runs an accelerated course in mice fasted for forty-eight hours, r subjected to various types of metabolic disturbances. On the other hand, we have found hat many of the regimens which enhance bacterial infections also increase the susceptibility f mice to the endotoxins of Gram-negative bacilli or of tubercle bacilli (Tables V and VI).

TABLE V.—EFFECT OF DIETARY REGIMENS ON SUSCEPTIBILITY OF MICE TO ENDOTOXIN OF *Kl. pneumoniæ*

Regimen	LD50 of toxin ml.
Sherman diet *ad lib.*	0·5
48 hours fasting before injection of toxin	0·1
Glucose, minerals, vitamins, gelatin*	0·05
Glucose, casein, gelatin*	0·4
Gl., min., vit., essential amino acids*	0·2

* These diets fed for 5 days before injection of toxin.

TABLE VI.—EFFECT OF DIETARY REGIMENS ON SUSCEPTIBILITY OF MICE TO TOXICITY OF PHENOL-KILLED TUBERCLE BACILLI

Mice fasted forty-eight hours, then fed following diets *ad lib.* for five days before test	Mortality caused by toxin	
	5 mg. in saline %	0·5 mg. in oil %
Glucose, vitamins, gelatin	90	100
,, ,, essential amino acids	75	—
,, ,, gelatin, casein	20	0
,, ,, ,, ,, citrate	60	—
Sherman diet	0	0

It is clear therefore that metabolic disturbances can modify the infectious process by affecting determinants of the host-parasite relationship other than the bactericidal power of tissues.

Whatever its mechanism, the increase in susceptibility of mice to infection brought about by fasting is transient, the animals recovering their normal state of resistance forty-eight hours after being provided pellets or Sherman diet *ad lib.* This remarkable reversibility of the infection-enhancing effect of fasting provides an experimental approach to the study of the nutritional determinants of susceptibility and resistance. Some of the preliminary results so far obtained permit the following general statements.

Animals given glucose *ad lib.* either in the drinking fluid, or in gelatin cakes, with no other dietary constituent, proved more susceptible to infection than those receiving water or saline alone. Susceptibility was even greater when the glucose was supplemented with a complete salt mixture, inositol, all the known vitamins (water-soluble and fat-soluble), oil, and a number of different amino acids. Similarly, feeding casein alone, or casein with minerals and a complete vitamin mixture, failed to correct the effect of fasting. Susceptibility was always high when casein was replaced by a mixture of all essential amino acids in the diet even when the latter contained all other known essential growth factors. As far as can be judged at the present time, the metabolic disturbances which increased the susceptibility of mice to bacterial infection also increased their susceptibility to endotoxin.

DISCUSSION

We shall not attempt to discuss here the biochemical reactions through which metabolic and physiological disturbances can affect resistance to infection. But whatever the nature of the fundamental mechanisms responsible for this effect, some conclusions of biological interest appear justified.

It is clear that the cases of enhancement of infection described in this report are independent of ordinary immunological mechanisms. This is evident from the fact that huge differences can be recognized between the numbers of bacteria present in the tissues of normal and fasted mice within a few hours after injection of Friedländer bacilli—long before an immune response could have taken place.

We have repeatedly observed that, in mice subjected to metabolic disturbances, the liver is the organ which exhibits the most profound decrease in bactericidal power and the most extensive multiplication of bacteria. As is well known, there is much evidence that the bactericidal power of the body for bacteria, and its resistance to the endotoxins of Gram-negative bacilli, reside in the cells of the reticulo-endothelial system (Beeson, 1947). It is

not unlikely, therefore, that metabolic disturbances decrease resistance to infection through their effect on this system. The fact that the LD50 (by intravenous injection) of staphylococci and Friedländer bacilli can be dramatically reduced by subjecting mice to these disturbances suggests that the ability of the animals to dispose of injected bacteria is directly involved. Loss in the ability of the reticulo-endothelial cells to deal with bacterial toxins may also aggravate the toxic manifestations of the disease and indirectly activate the progress of the infection.

I recognize that the experimental infections used in this study, and the metabolic disturbances superimposed upon them, constitute artificial laboratory models which may not have any exact counterpart in disease as it occurs in Nature. Nevertheless, I believe that some of the findings which have been reported here have a bearing on epidemiological and pathological problems. They illustrate for example that susceptibility to infection can change rapidly and reversibly independently of the immunological state of the exposed individual, merely as a response to physiological stresses of the type encountered in everyday life. This knowledge provides an operational approach to the problem of "natural resistance" and points to the fact that infection and disease may be controlled by unrelated determinants.

It is well known that increased susceptibility to infection is the consequence of many forms of physiological misery. The individual depleted by trauma or shock resembles the fasted mouse in his reduced ability to restrain the multiplication of bacterial agents, or to overcome the action of their toxins (Fine, 1954). The findings in mice suggest that this depletion and the accompanying susceptibility may not be corrected by providing a large caloric intake or even by administering sugar-vitamins-amino-acid mixtures. The factors essential for growth, reproduction and other normal physiological processes are not necessarily the ones most important in determining receptivity to infectious agents and resistance to the diseases that they cause (*see*, for example, Table I). The two latter properties are controlled, not by what is good for the host considered as an isolated system, but rather by the determinants of host-parasite relationship. It is even possible that receptivity to infection, and resistance to disease, are two different characteristics with independent mechanisms.

Conclusions

The susceptibility of mice to experimental bacterial infections can be increased consistently by a variety of procedures designed to disturb animal metabolism.

These infection-enhancing procedures express themselves in higher morbidity and mortality, shorter survival time, larger bacterial populations in the various organs (particularly the liver), and increased susceptibility to endotoxins of Gram-negative bacilli and tubercle bacilli.

The infection-enhancing effect of fasting becomes manifest within thirty-six hours and can be rapidly corrected by feeding a complete natural diet *ad lib.* In contrast, it is further aggravated when glucose, or an incomplete diet, is given to the animal during the fasting period. It could not be corrected by feeding a diet containing sugar, fat, minerals, all known vitamins, and all essential amino acids, for five days before challenge with living bacteria or toxin.

These findings are discussed with reference to their biological mechanisms and to their bearing on various epidemiological and pathological problems.

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Professor A. A. Miles: I want to comment on two aspects of the analysis of the infective lesion so admirably discussed by Dr. Dubos; firstly, the importance of study of the early stages of infection, and secondly the importance of the "physiological" as well as of "biochemical" analysis. The early stage deserves especial study because reactions in this period determine everything—local suppression, extension or generalization of the infection—that follows the primary lodgement. The later stages, when characteristic lesions are established, tell us only what the body does to win the second round of the contest, obviously having lost the first round in the primary lodgement and having permitted the invaders to get a foothold.

The physiology of the early stages at present is largely concerned with the vascular consequences of local infection. When washed suspensions of moderately virulent bacteria are injected intradermally in the guinea-pig, they produce their maximum effect in twenty-four to forty-eight hours in local lesions whose size is dependent on the dose. These local

infections can be enhanced by the simultaneous injection of various drugs. Of those drugs adrenaline is one of the most useful, since it can be used in doses that have no effect on the bacteria *in vitro*, or on the bactericidal action of blood, and that do no lasting damage to the tissue. A dose of 2 μg., which grossly diminishes the local blood supply for two and a half hours, enhances the infectivity of bacteria from 10- to 10 million-fold, according to the species and strain tested. The degree of enhancement is not important as such, but a deduction of some importance may be made from it. Thus, a 100-fold enhancement indicates that the local defensive reactions that are inhibited by a simple two and a half hours decrease in blood supply, are clearly worth a 99% "kill" of the bacteria introduced.

By a slightly different intradermal technique, we can measure the period during which this local initial "kill" is most effective. A series of injections is made, and the adrenaline injected into the same sites when the lesions are 0, 1, 2, 3 . . . &c. hours old. The lesion finally developing at the site given simultaneous adrenaline and bacteria is greatly enhanced; but lesions that are four hours old at the time of adrenaline superinjection prove to be insusceptible to adrenaline enhancement. In other words, there is a decisive period of four to five hours when this initial "kill" takes place; the ultimate maximum size of the lesion is determined at this stage. This decisive period of four to five hours or less obtains with, e.g., Group C streptococcus, *Staph. aureus*, *C. diphtheriæ*, *C. ovis*, *Cl. welchii* and *E. coli*. As my former colleague Dr. A. C. Dutton has shown (unpublished), the same phenomenon occurs in mice subcutaneously infected with bacteria that ultimately produce bacteriæmic death. Thus, adrenaline enhances the ultimately lethal effect of subcutaneous Group C streptococcus to well over 100-fold; but, put into local streptococcal lesions when they are four hours old, it has no effect. Here again, the local reaction, which determines death about five days later, is decisively effective within the first four hours.

The same decisive period is found when other local modifiers are used, and when local skin lesions are enhanced by the induction of generalized shock. Thus, lesions initiated during a period of shock are greatly enhanced; the final size of lesions that are four hours old before shock is induced is not affected by the shock. The uncovering of a highly effective "kill" in early bacterial lesions and of a decisive period was made with rapidly growing organisms—e.g. streptococci, staphylococci, coliforms, clostridia, corynebacteria—all of which in their various ways are rapidly dealt with by the anti-bacterial substances and cells in the tissues. With slowly growing organisms, which are less immediately susceptible, the effects are different. With the vole bacillus, e.g. whose lesions come to maturity in ten days, the "kill" in the first few hours is 60–70%, and the decisive period is longer than eight hours. Nevertheless, a dose of the anti-complementary substance liquoid, whose effect lasts only a few hours in the tissues, is sufficient to enhance the infectivity of the vole bacillus, estimated in lesions read ten days later, by well over 50-fold.

When attempts are made to enhance the vole bacillus with liquoid in the allergic animal, at ten days the lesions, compared with those in the normal animal, are enhanced to a degree far greater than that produced by liquoid. This is evidence, as far as the local lesion is concerned, of the ill-effects of allergy in defence. A further analysis, however, is possible. Tuberculin allergy has an acute anaphylactic component, in addition to the delayed reaction. When tuberculin is injected, there is an immediate increase in capillary permeability, which in amount and duration is very like that induced by histamine and by globulin permeability factors (Wilhelm *et al.*, 1955) present in guinea-pig serum. We may suppose that when tubercle bacilli make their primary lodgement in an allergic animal, in addition to promoting the disadvantageous delayed type of reaction, they might promote an immediate exudation of blood fluids; and this exudate might be useful in defence. The point can be tested by measuring the effect of small, permeability-increasing doses of histamine, or of the serum permeability factor, on the results of intradermal injections of the vole bacillus in normal animals. When this is done a 3- to 5-fold depression of infectivity occurs. It cannot at present be said whether this is due to the exudation of bactericidal substances acting locally, or to the removal of the injected bacilli to the lymphatics and the circulation, as a result of increased movement of fluid in the tissues. Nevertheless, as a result of such immediate anaphylactic type of exudation, there is apparently a 67–80% "kill" of the injected bacteria (Miles, 1955). This is not numerically a very striking result, but it is not incompatible with the view that in a very restricted zone of circumstances, the primary lodgement of tubercle bacilli in allergic tissues might be removed or destroyed more rapidly than in normal tissues; and this would be beneficial, provided that the local survivors were not numerous enough to take advantage of the decrease of resistance that delayed allergy apparently imposes on the tissues.

References

Miles, A. A. (1955) In. Ciba Foundation Symposium on Experimental Tuberculosis. (Eds. G. E. W. Wolstenholme and M. P. Cameron) London: p. 87.
Wilhelm, D. L., Miles, A. A., and Mackay, M. E. (1955) *Brit. J. exp. Path.*, **36**, 82.

120a

dissolving 99.5 mg. of $Na_4Fe(CN)_6 \cdot 10H_2O$ in 500.0 ml. of water, then transfer 1.0 ml. of this solution into a similar 150-ml. container for the control. To each container add 2 ml. of ferrous sulfate T.S. and 1 ml. of diluted sulfuric acid T.S., dilute to 100 ml. with water, and mix. Transfer 50-ml. portions of the respective solutions into matched color-comparison tubes. The sample solution shows no more blue color than the control.

Arsenic. A solution of 1 gram in 10 ml. of water meets the requirements of the *Arsenic Test*, page 720.

Heavy metals. A solution of 4 grams in 25 ml. of water meets the requirements of the *Heavy Metals Test*, page 763, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Loss on drying, page 774. Dry at 105° for 2 hours.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Nutrient; preservative; flavoring agent and intensifier.

SODIUM CITRATE

$C_6H_5Na_3O_7 \cdot 2H_2O$ Mol. wt. 294.10

DESCRIPTION

Sodium citrate is anhydrous or contains two molecules of water of crystallization. It occurs as colorless crystals or as a white, crystalline powder. One gram of the dihydrate dissolves in 1.5 ml. of water at 25° and in 0.6 ml. of boiling water. It is insoluble in alcohol. A 1 in 20 solution gives positive tests for *Sodium*, page 771, and for *Citrate*, page 769.

SPECIFICATIONS

Assay. Not less than 99 per cent of $C_6H_5Na_3O_7$, calculated on the anhydrous basis.

Water. *Anhydrous sodium citrate*, not more than 1 per cent; *sodium citrate dihydrate*, between 10 and 13 per cent.

Limits of Impurities

Alkalinity. Passes test.

Arsenic (as As). Not more than 3 parts per million (0.0003 per cent).

Heavy metals (as Pb). Not more than 10 parts per million (0.001 per cent).

TESTS

Assay. Transfer about 350 mg., accurately weighed, to a 250-ml. beaker. Add 100 ml. of glacial acetic acid, stir until completely dissolve and titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the end-point potentiometrically. Each ml. of 0.1 *N* perchloric acid is equivalent to 8.602 mg. of $C_6H_5Na_3O_7$.

Water. Determine by drying at 180° for 18 hours (see page 774), or by the *Karl Fischer Titrimetric Method*, page 804.

Alkalinity. A solution of 1 gram in 20 ml. of water is alkaline to litmus paper, but after the addition of 0.2 ml. of 0.1 *N* sulfuric acid no pink color is produced by 1 drop of phenolphthalein T.S.

Arsenic. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 720.

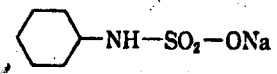
Heavy metals. A solution of 2 grams in 25 ml. of water meets the requirements of the *Heavy Metals Test*, page 763, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Packaging and storage. Store in tight containers.

Functional use in foods. Buffer; sequestrant; nutrient for culture buttermilk.

~~SODIUM CYCLAMATE~~

Sodium Cyclohexanesulfamate



$C_6H_{12}NNaO_3S$ Mol. wt. 201.2

DESCRIPTION

White, odorless crystals or crystalline powder. In dilute solution it is about 30 times as sweet as sucrose. One gram is soluble in 5 ml. of water and in about 24 ml. of propylene glycol. It is practically insoluble in alcohol, in chloroform, and in ether.

IDENTIFICATION

A. To 10 ml. of a 1 in 100 solution add 1 ml. of hydrochloric acid, mix, then add 1 ml. of barium chloride T.S. The solution remains clear, but upon the addition of 1 ml. of sodium nitrite solution (1 in 10) a white precipitate forms.

B. A 1 in 100 solution gives positive tests for *Sodium*, page 771.

SPECIFICATIONS

Assay. Not less than 98 per cent and not more than the equivalent of 101 per cent of $C_6H_{12}NNaO_3S$.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per mi (0.0003 per cent).

120a

Lead. A solution of 1 gram in 20 ml. of water meets the requirements of the *Lead Limit Test*, page 772, using 10 mcg. of lead ion (Pb) in the control.

Magnesium and alkali salts. Dissolve 1 gram in about 50 ml. of water, add 500 mg. of ammonium chloride, mix, and boil for about 1 minute. Rapidly add 40 ml. of oxalic acid T.S., and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red T.S., then add ammonia T.S., dropwise, until the mixture is just alkaline, and cool. Transfer the mixture into a 100-ml. cylinder, dilute with water to 100 ml., let it stand for 4 hours or overnight, and then decant the clear, supernatant liquid through a dry filter paper. To 50 ml. of the clear filtrate in a platinum dish add 0.5 ml. of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 20 mg.

Packaging and storage. Store in tight containers.

Functional use in foods. Miscellaneous and general purpose; sequestrant; firming agent.

CALCIUM CITRATE

$Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$ Mol. wt. 570.51

DESCRIPTION

A fine, white, odorless powder. It is very slightly soluble in water and insoluble in alcohol.

IDENTIFICATION

A. Dissolve 500 mg. in 10 ml. of water and 2.5 ml. of diluted nitric acid T.S., add 1 ml. of mercuric sulfate T.S., heat to boiling and then add potassium permanganate T.S. A white precipitate is formed.

B. Ignite 500 mg. completely at as low a temperature as possible, cool, and dissolve the residue in 10 ml. of water and 1 ml. of glacial acetic acid. Filter and add 10 ml. of ammonium oxalate T.S. to the filtrate. A voluminous white precipitate appears which is soluble in hydrochloric acid.

SPECIFICATIONS

Assay. Not less than 97.5 per cent of $Ca_3(C_6H_5O_7)_2$ after drying.

Loss on drying. Between 10 and 13.3 per cent.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 per cent).

Fluoride. Not more than 30 parts per million (0.003 per cent).

Heavy metals (as Pb). Not more than 20 parts per million (0.002 per cent).

Lead. Not more than 10 parts per million (0.001 per cent).

TESTS

Assay. Dissolve about 350 mg., previously dried at 150° to constant weight and accurately weighed, in a mixture of 10 ml. of water and 2 ml. of diluted hydrochloric acid T.S., and dilute to about 100 ml. with water. While stirring, preferably with a magnetic stirrer, add about 30 ml. of 0.05 *M* disodium ethylenediaminetetraacetate from a 50-ml. buret, then add 15 ml. of sodium hydroxide T.S. and 300 mg. of hydroxy naphthol blue indicator, and continue the titration to a blue end-point. Each ml. of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to 8.303 mg. of $Ca_3(C_6H_5O_7)_2$.

Loss on drying, page 774. Dry to constant weight at 150°.

Arsenic. A solution of 1 gram in 5 ml. of diluted hydrochloric acid T.S. meets the requirements of the *Arsenic Test*, page 720.

Fluoride. Weigh accurately 1.67 grams, and proceed as directed in the *Fluoride Limit Test*, page 762.

Heavy metals. Dissolve 1 gram in 20 ml. of water and 2 ml. of hydrochloric acid, add 1.5 ml. of stronger ammonia T.S., and dilute to 25 ml. with water. This solution meets the requirements of the *Heavy Metals Test*, page 763, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

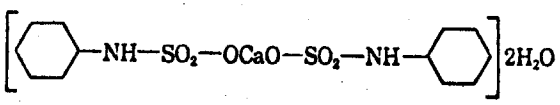
Lead. Dissolve 1 gram in 10 ml. of water and 1 ml. of hydrochloric acid. This solution meets the requirements of the *Lead Limit Test*, page 772, using 10 mcg. of lead ion (Pb) in the control.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Sequestrant; buffer; firming agent

~~CALCIUM CYCLAMATE~~

Calcium Cyclohexanesulfamate



$C_{12}H_{24}CaN_2O_6S_2 \cdot 2H_2O$ Mol. wt. 432.58

DESCRIPTION

White odorless crystals or crystalline powder. In dilute solutions it is about 30 times as sweet as sucrose. Its solutions are neutral to litmus. One gram is soluble in about 4 ml. of water, in about [illegible] ml. of propylene

meets the requirements of the
of lead ion (Pb) in the control.

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HLORIDE

Mol. wt. 74.56

ical crystals, or a white, granu-
taste, and is stable in air. Its
m dissolves in 2.8 ml. of water
ter is insoluble in alcohol.
Potassium, page 771, and for

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ts per million (0.0003 per cent).
nan 10 parts per million (0.001

er cent.

2 hours, weigh accurately, and
pered flask. Add, while agitat-
ml. of nitric acid, and 5 ml. of
ml. of ferric ammonium sulfate
e with 0.1 *N* ammonium thio-
e is equivalent to 7.456 mg. of

of 5 grams in 50 ml. of recently
phenolphthalein T.S. No pink
0.02 *N* sodium hydroxide. A

ml. of water meets the require-

120a

Heavy metals. A solution of 2 grams in 25 ml. of water meets the requirements of the *Heavy Metals Test*, page 763, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Loss on drying, page 774. Dry at 105° for 2 hours.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Nutrient; dietary supplement.

POTASSIUM CITRATE

$C_6H_5K_3O_7 \cdot H_2O$ **Mol. wt. 324.42**

DESCRIPTION

Transparent crystals, or a white, granular powder. It is odorless, has a cooling, saline taste, and is deliquescent when exposed to moist air. One gram dissolves in about 0.5 ml. of water. It is almost insoluble in alcohol. A 1 in 10 solution gives positive tests for *Potassium*, page 771, and for *Citrate*, page 769.

SPECIFICATIONS

Assay. Not less than 99 per cent of $C_6H_5K_3O_7$ after drying.

Loss on drying. Between 3 per cent and 6 per cent.

Limits of Impurities

Alkalinity. Passes test.

Arsenic (as As). Not more than 3 parts per million (0.0003 per cent).

Heavy metals (as Pb). Not more than 10 parts per million (0.001 per cent).

TESTS

Assay. Dissolve about 250 mg., previously dried at 180° for 4 hours and accurately weighed, in 40 ml. of glacial acetic acid, warming slightly to effect solution. Cool the solution to room temperature, add 2 drops of crystal violet T.S., and titrate with 0.1 *N* perchloric acid. Perform a blank determination (see page 2) and make any necessary correction. Each ml. of 0.1 *N* perchloric acid is equivalent to 10.214 mg. of $C_6H_5K_3O_7$.

Loss on drying, page 774. Dry at 180° for 4 hours.

Alkalinity. A 1 in 20 solution is alkaline to litmus, but after the addition of 0.2 ml. of 0.1 *N* sulfuric acid to 10 ml. of this solution no pink color is produced by the addition of 1 drop of phenolphthalein T.S.

Arsenic. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 720.

Heavy metals. A solution of 2 grams in 25 ml. of water meets the requirements of the *Heavy Metals Test*, page 763, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Packaging and storage. Store in tight containers.

Functional use in foods. Miscellaneous and general purpose; buffer; sequestrant.

120a

Specific gravity. Between 1.034 and 1.037 at 20°.

Limits of Impurities

Acidity (as acetic acid). Not more than 0.04 per cent.

Arsenic (as As). Not more than 3 parts per million (0.0003 per cent).

Heavy metals (as Pb). Not more than 10 parts per million (0.001 per cent).

TESTS

Assay. Weigh accurately about 1.5 grams, and proceed as directed under *Ester Determination*, page 742, using 50.40 as the equivalence factor (*e*) in the calculation.

Specific gravity. Determine by any reliable method (see page 4).

Acidity. To 75 ml. of methanol, contained in a 250-ml. wide-mouth Erlenmeyer flask, add 1 ml. of bromothymol blue T.S., and titrate with 0.05 *N* sodium hydroxide to a blue end-point. Transfer a 20.0-ml. sample into the flask, calculate its weight from its specific gravity, and titrate back to the same blue end-point. Each ml. of 0.05 *N* sodium hydroxide is equivalent to 3 mg. of acetic acid ($C_2H_4O_2$).

Arsenic. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 720.

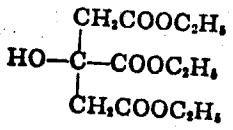
Heavy metals. Prepare and test a 2-gram sample as directed in *Method II* under the *Heavy Metals Test*, page 763, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Packaging and storage. Store in tight containers.

Functional use in foods. Flavoring agent.

TRIETHYL CITRATE

Ethyl Citrate



$C_{12}H_{20}O_7$

Mol. wt. 276.29

DESCRIPTION

An odorless, practically colorless, oily liquid. It is slightly soluble in water, but is miscible with alcohol and with ether. Its specific gravity is approximately 1.137 at 20°.

SPECIFICATI

Assay. Not less than 99 per cent of $C_{12}H_{20}O_7$.

Refractive index. Between 1.439 and 1.441.

Limits of Impurities

Acidity (as citric acid). Not more than 0.02 per cent.

Arsenic (as As). Not more than 3 parts per million (0.0003 per cent).

Heavy metals (as Pb). Not more than 10 parts per million (0.001 per cent).

Water. Not more than 0.25 per cent.

TESTS

Assay. Weigh accurately about 1.5 grams of the sample into a 500-ml. flask equipped with a standard taper ground joint, and add 25 ml. of isopropyl alcohol and 25 ml. of water. Pipet 50 ml. of 0.5 *N* sodium hydroxide into the mixture, add a few boiling chips, and attach a suitable water-cooled condenser. Reflux for 1.5 hours, then cool, wash down the condenser with about 20 ml. of water, add 5 drops of phenolphthalein T.S., and titrate the excess alkali with 0.5 *N* sulfuric acid. Perform a blank determination (see page 2). Each ml. of 0.5 *N* sulfuric acid is equivalent to 46.05 mg. of $C_{12}H_{20}O_7$.

Refractive index, page 785. Determine with an Abbé or other refractometer of equal or greater accuracy.

Acidity. Dissolve 3.2 grams, accurately weighed, in 20 ml. of neutralized alcohol, add phenolphthalein T.S., and titrate with 0.1 *N* sodium hydroxide. Not more than 10.0 ml. is required.

Arsenic. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 720.

Heavy metals. Prepare and test a 2-gram sample as directed in *Method II* under the *Heavy Metals Test*, page 763, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Water. Determine by the *Karl Fischer Titrimetric Method*, page 804.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Miscellaneous; general purpose.

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pertaining to specifica-
e sources.

2

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parts per million (0.001

per cent.
1.5 per cent.

Method I in the general

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Test, page 720.
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53, using 20 mcg. of lead

hod, page 468.
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mine as directed in the

metric Method, page 804.
d containers.
d manufacture of other

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STEARYL MONOGLYCERIDYL CITRATE

DESCRIPTION

A soft, practically tasteless, off-white to tan, waxy solid having a lard-like consistency. It is insoluble in water but is soluble in chloroform and in ethylene glycol. It is prepared by a controlled chemical reaction from citric acid, monoglycerides of fatty acids (obtained by the glycerolysis of edible fats and oils or derived from fatty acids), and stearyl alcohol. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources.

SPECIFICATIONS

Acid value. Between 40 and 52. meq/kg

Total citric acid. Between 15 and 18 per cent.

Saponification value. Between 215 and 255.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 per cent).

Heavy metals (as Pb). Not more than 10 parts per million (0.001 per cent).

Residue on ignition. Not more than 0.1 per cent.

Water. Not more than 0.25 per cent.

TESTS

Acid value. Determine as directed under *Method II* in the general procedure, page 748.

Total citric acid

Brominating Solution. Dissolve 19.84 grams of potassium bromide, 5.44 grams of potassium bromate, and 12 grams of sodium metavanadate, $NaVO_3$, in water by warming, and dilute to 1000 ml. with water. Filter if necessary.

Ferrous Sulfate Solution. Dissolve 44 grams of ferrous sulfate, $FeSO_4.7H_2O$, in 1 *N* sulfuric acid, dilute to 100 ml. with 1 *N* sulfuric acid, and mix. Use within 5 days of preparation.

Sulfide Solution. On the day of use, dissolve 4 grams of thiourea in 100 ml. of a 1 in 50 solution of sodium borate, $Na_2B_4O_7.10H_2O$, and add 2 ml. of sodium sulfide T.S. Wait 30 minutes after the addition of the sodium sulfide T.S. before using.

Standard Solution. Transfer about 50 mg. of sodium citrate dihydrate, accurately weighed, into a 500-ml. volumetric flask, dissolve and dilute to volume with water, and mix. Transfer 15.0 ml. of this solution into a 100-ml. volumetric flask, dilute to volume with water, and mix. Calculate the concentration (*C*), in mcg. per ml., of citric acid in

the final solution by the formula $(15 \times 1000 \times 0.6533W)/(100 \times 500)$, in which W is the weight, in mg., of the sodium citrate taken, and 0.6533 is the factor converting sodium citrate dihydrate to citric acid.

Sample Solution. Transfer about 250 mg. of the sample, accurately weighed, to a 250-ml. extraction flask, and add 15 ml. of 0.5 N sodium hydroxide, 5 ml. of alcohol, and a few glass beads. Connect the flask with a water-cooled condenser, and reflux for 3 hours. Immediately cool and neutralize to phenolphthalein T.S. with 0.5 N hydrochloric acid, then place the flask in an ice-bath and add 5 ml. of sulfuric acid T.S. Transfer the solution to a 125-ml. separator, extract with three 40-ml. portions of chloroform, and then extract the combined chloroform extracts in a 250-ml. separator with three 10-ml. portions of 0.5 N sulfuric acid, adding the acid extracts to a second 250-ml. separator. Wash the combined acid extracts with two 60-ml. portions of chloroform, and discard the chloroform washes. Filter the acid solution into a 500-ml. volumetric flask, neutralize slowly with 6 N sodium carbonate, and dilute to volume with water. Transfer 10.0 ml. of this solution into a 100-ml. volumetric flask, dilute to volume with water, and mix. Each ml. of the final solution contains approximately 10 mcg. of citric acid.

Procedure. Pipet 2 ml. each of the *Standard Solution* and of the *Sample Solution* into separate 40- or 45-ml. glass-stoppered centrifuge tubes, and add 3 ml. of water to each tube. Place 5 ml. of water in a third tube for the reagent blank. Place the tubes in an ice-bath, add 5 ml. of sulfuric acid T.S., mix thoroughly, and allow to stand for exactly 5 minutes. Remove the tubes from the ice-bath, and allow them to come to room temperature during the next 5 minutes. To each tube add 5 ml. of the *Brominating Solution*, then insert the stoppers, invert the tubes once or twice, and heat in a water bath at 30° for 20 minutes. Remove the tubes, add 1.5 ml. of *Ferrous Sulfate Solution*, invert again, and allow to stand for 5 minutes, shaking occasionally to ensure complete reduction of the excess free bromine in the tubes. Add 6.5 ml. of petroleum ether, shake for 2 or 3 minutes, and remove the water layer with a syringe. Wash the ether solutions with 15 ml. of water, then remove the water and filter the ether extracts into the original centrifuge tubes which have been previously rinsed with the *Sulfide Solution*. Filter each ether extract through a tight plug of glass wool onto which has been placed a sufficient amount of anhydrous sodium sulfate to remove the last traces of water from the ether. Place 5.0 ml. of the filtrate in a clean, dry centrifuge tube, add 3 ml. of *Sulfide Solution*, shake vigorously for 1.5 minutes, and centrifuge. Decant about 0.5 ml. of the supernatant ether layer from each tube, then carefully transfer the ether solutions into 1-cm. cells and determine the absorbance of the extracts obtained from the *Standard Solution* and the *Sample Solution* at 500 mμ with a suitable spectrophotometer, using the reagent blank in the reference cell. Calculate the quantity, in mg., of citric acid in the sample taken by the formula $5C \times A_U/A_S$, in which C is the exact concentration, in mg. per ml., of citric acid in the *Standard Solution*, A_U is the absorbance of the solution from the *Sample Solution*, and A_S is the absorbance of the solution from the *Standard Solution*.

Saponification value. Transfer about 1 gram, accurately weighed, into a 250-ml. Erlenmeyer flask, and add 25 ml. of ethylene glycol, 35.0 ml. of 0.5 N alcoholic potassium hydroxide, and a few glass beads. Reflux for one hour, using a water condenser, then rinse the condenser with water and cool. Add 1 ml. of phenolphthalein T.S., and titrate with 0.5 N hydrochloric acid. Perform a blank determination (see page 2) but do not reflux. The difference between the volumes, in ml., of 0.5 N hydrochloric acid consumed in the actual test and in the blank titration, multiplied by 28.05 and divided by the weight, in grams, of the sample taken, is the saponification value.

Arsenic. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 720.

Heavy metals. Prepare and test a 2-gram sample as directed in *Method II* under the *Heavy Metals Test*, page 763, using 20 mcg. of lead ion (Pb) in the control.

Residue on ignition. Ignite 1 gram as directed in the general procedure, page 786.

Water. Determine by the *Karl Fischer Titrimetric Method*, page 804.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Emulsion stabilizer.

~~SUCCINIC ACID~~

Butanedioic Acid

$HOOCCH_2CH_2COOH$

$C_4H_6O_4$ Mol. wt. 118

DESCRIPTION

Colorless or white, odorless crystals having an acid taste. One gr dissolves in 13 ml. of water at 25°, in 1 ml. of boiling water, in 18.5 of alcohol, and in 20 ml. of glycerin.

SPECIFICATIONS

Assay. Not less than 99 per cent of $C_4H_6O_4$.

Melting range. Between 185° and 190°.

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14663

Effect of Parenterally Administered Citrate on the Renal Excretion of Calcium.*

GEORGE GOMORI AND ELSIE GULYAS.

From the Department of Medicine, The University of Chicago.

There is an increasing body of evidence for the importance of citric acid in the metabolism of calcium. Shohl and Butler[1] found that in the treatment of infantile rickets citrate has a vitamin D-like action. Dickens[2] reported that bone contains as much as 1.5% citric acid, and that the citrate content of bone is increased by the administration of parathormone. Letonoff and Kety[3] showed that citric acid mobilizes lead from the bones. Scott, Huggins and Selman[4] confirmed previous findings that the renal excretion of citrate is decreased in patients suffering from urolithiasis.

In the experiments to be reported here the effect of the parenteral administration of sodium citrate on the renal excretion of calcium and phosphorus and on the histology of bones was studied.

Experimental. The animals used were 7 adult dogs, weighing 10 to 15 kg, 4 puppies and 6 rats.

1. The adult dogs had cannula-cystostomies performed upon them about 1 week before the experiment. They were fasted for 17 hours. On the morning of the experiment they were fastened to a device essentially similar to that described by Huggins and Clark[5] and kept standing on their feet during the periods of urine collection. At 9:00 a. m. they were given about 200 cc of water by stomach tube. Flasks were attached to and removed from the cannula at 30 minute intervals from 9:30 on; specimens were collected at 10:00, 10:30, 11:00, 11:30, and finally one for the interval from 4:00 to 4:30 p. m. At 10:30 100 cc of a 4% solution of sodium citrate was injected subcutaneously. No reactions of any kind were observed. Blood samples were taken at 9:30 and 11:30 a. m. and at 4:30 p. m. Calcium was determined by the Clark-Collip[6] modification of the Kramer-Tisdall[7] method; inorganic phosphorus by the photoelectric elon method.[8] The urine samples were ashed.

Table I shows the results in 4 of the animals. In the remaining 3 animals the results were essentially similar. Urinary calcium and phosphorus are given in 30-minute totals.

As shown in the table, the injection of citrate is followed by a rapid increase in the rate of renal elimination of calcium. A high level of excretion is reached after 60 minutes which later declines, and 5 hours after the injection it is practically back to its initial level. The excretion of phosphate does not follow any typical course, except for a tendency to a late increase in excretion. The blood levels of both calcium and phosphate remain essentially unchanged throughout. The marked increase in the renal clearance of calcium is obviously due to the fact that a larger fraction of the plasma calcium becomes ultrafiltrable.

2. Two puppies (A and B), weighing 1500 and 3000 g, respectively, were put in individual metabolism cages and fed a standard diet. For a control period of 2 days they were left untreated; for the following 2 days they received 30 cc/kg of normal saline subcutaneously, divided into a morning and an evening dose; for the last 2 days they were given 30 cc/kg of 4% sodium citrate in the same way. The daily amounts of urine were analyzed for calcium and phosphorus.

Table II shows the results.

It is clearly visible that on the citrate days the excretion of calcium was considerably increased.

Puppy B was found dead in its cage on the morning of the eighth day; autopsy revealed pulmonary edema and some edema of the subcutaneous tissues throughout the body. The microscopic changes in the bones will be described under experiment 3.

3. Puppy C, weighing 1300 g, was given 2 daily injections of 7 cc of 4% sodium citrate each; Puppy D, weighing 1500 g, was given 2 daily injections of 15 cc each. On the afternoon of the third day biopsies of the tibial crests were performed. Microscopic examination of the specimens showed hyperemia

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TABLE I.
Effect of Citrate on Urinary Excretion of Ca and P.

Time	4% citrate inj.	Urinary Ca 30 min. (mg)	Urinary P 30 min. (mg)	Serum Ca mg %	Serum P mg %	4% citrate inj.	Urinary Ca 30 min. (mg)	Urinary P 30 min. (mg)	Serum Ca mg %	Serum P mg %
	Dog 509					Dog 602				
9:30 AM		—	—	11	3.9		—	—	10	3.5
10:00		0.36	1.96				0.52	0.54		
10:30	100 cc	0.31	0.44			100 cc	0.57	0.85		
11:00		0.64	0.30				1.04	0.83		
11:30		9.0	9.1	11	3.7		1.78	0.83	10	5.5
4:30 P.M.		0.59	15.5	11	3.8		0.42	2.0	9	6.4
	Dog 213					Dog 188				
9:30 AM		—	—	10	3.7		—	—	10	5.1
10:00		0.70	2.87				0.49	3.3		
10:30	100 cc	0.77	0.39			100 cc	0.34	6.9		
11:00		3.7	0.42				1.4	8.3		
11:30		10.0	7.1	10	3.1		7.8	7.8	12	4.0
4:30 PM		0.55	1.86	11	4.6		0.36	7.3	9	3.9

TABLE II.
Urinary Excretion of Ca and P in 2 Puppies Under Control Conditions and Under Citrate Treatment.

Day	Treatment	Urinary excretion (mg/24 hours) Ca: Puppy A	Ca: Puppy B	P: Puppy A	P: Puppy B
1	None	0.36	15	23	110
2	None	0.88	31	47	120
3	30 cc saline/kg	0.71	25	86	104
4	30 " " "	2.0	15	78	84
5	30 " citrate/kg	20	75	90	174
6	30 " " "	30	64	73	155

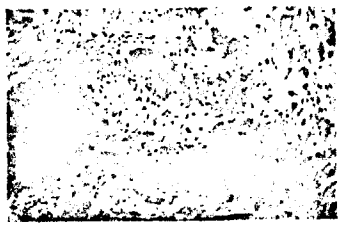

FIG. 1.
Osteoclastic absorption of bone and fibrosis of bone marrow in the upper tibial metaphysis of Puppy B.

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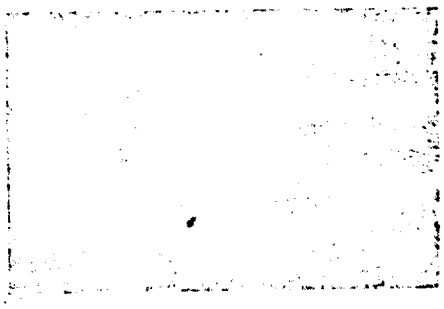

FIG. 2.
Similar changes in the rib of a citrate-treated rat, close to the costochondral junction.

and edema of the bone marrow, transformation of osteoblasts into spindle cells, an increase in the numbers of osteoclasts far beyond the limits of normal, fragmentation of bone trabeculae, and areas of early fibrosis—a picture extremely similar to that seen after toxic doses of parathormone. Very similar but even more severe changes were seen in the metaphysis of the tibia and in the costochondral junctions of Puppy B.

4. Three rats, weighing 100 g each, and 3 others, weighing 50 g each, were given subcutaneously a daily amount of 60 cc/kg of a 4% sodium citrate solution, divided in 2 doses, for 5 days. After 5 days the animals were killed and their bones examined microscopically. The bones of the heavier animals did not show clear-cut changes, while the bones, especially the ribs, of the smaller animals presented lesions essentially identical with those seen in the puppies, although the changes in the rats were much milder.

Summary. The subcutaneous injection of 8 to 30 cc/kg of a 4% solution of sodium citrate into dogs causes a considerable and prompt increase in the urinary excretion of calcium while leaving the blood calcium level essentially unaffected. Repeated subcutaneous injections of citrate into puppies and young rats lead to osseous changes very similar to those produced by large doses of parathormone.

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Management of Acute Myocardial Infarction by Narcosis.

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The relief of pain, anguish, and apprehension is a very important objective in the management of the initial phase of acute coronary occlusion and myocardial infarction. Morphine and barbiturates are commonly relied upon to secure this, but fail to do so in a satisfactory manner in a number of patients. Even after the abatement of pain, the exhibition of extreme restlessness with much tossing about after repeated administration of these drugs in therapeutic doses indicate the pressing need for some departure in the present almost standardized scheme of therapy. The frequent persistence of a considerable degree of excitement is perhaps more damaging than pain in raising the metabolic activity of the tissues and in imposing an additional burden on an already injured heart at a most critical period. It is therefore proposed on theoretical grounds, that the induction of a sufficiently deep narcosis for an arbitrary period of several days is worthy of trial in selected cases of this disease, notwithstanding certain hazards likely to be entailed by the procedure. By its adoption, however, the prime therapeutic desideratum, namely, that of reducing body metabolic activity, is immediately accomplished, and maintained over the period of narcosis. Three factors operating in this direction are: (1) the relaxation of the body musculature; (2) the almost total deprivation of food; and (3) the abolition of pain and apprehension. It is conceivable that during the prolonged period of sleep, there

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A STUDY ON THE COMPARATIVE TOXIC EFFECTS OF CITRIC ACID AND ITS SODIUM SALTS

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Since the introduction of citrated blood for transfusion in 1915 by Lewisohn (1), there has been considerable study as to its value and its disadvantages. In the original work, the relative safety of the procedure was demonstrated first in dogs and then in man. Following this, much was done to improve the technique and thereby reduce the number and severity of the untoward reactions. In recent years under the stimulus of massive transfusions of citrated blood for treatment of extreme shock produced by hemorrhage, there has been much study and speculation as to the occurrence of citrate intoxication (2). However, there apparently have been no comparative studies as to the toxicity of citric acid and its sodium salts as measured by the LD_{50}, although anticoagulant acid citrate dextrose solution is U.S.P. The following work was undertaken to determine the LD_{50} for citric acid as well as for its sodium salts.

METHOD. White mice, albino rats, rabbits and dogs were used as experimental animals. Equal molecular concentrations of citrate in the forms of citric acid, monosodium citrate, disodium citrate, and trisodium citrate were compared. Five hundred and ninety-nine intraperitoneal injections of 0.0477 Molar solutions were made in four hundred and seventy-seven mice weighing between 14 and 28 grams with an average weight of 19 grams. Three hundred and eighty-four intraperitoneal injections of 0.381 Molar solutions were made in two hundred and ninety-nine rats weighing between 85 and 170 grams with an average weight of 120 grams. Three hundred and sixty-one intravenous injections of 0.0119 Molar solutions were made into the tail veins of two hundred and seventy-nine mice weighing between 14.5 and 28.5 grams with an average weight of 22 grams and in these the whole dose was given within a few seconds. One hundred and fifty-eight intravenous injections of 0.477 Molar solutions were made into the lateral ear veins of one hundred rabbits weighing between 1.75 and 2.75 kilograms with an average weight of 1.95 kilograms. Here a constant injection rate of 0.75 cc./minute (0.358 millimoles/minute) was used. The LD_{50} was determined with each method of administration for citric acid, monosodium citrate, disodium citrate, and trisodium citrate by the method of Reed and Muench (3).

Eighty intravenous injections of 0.25 Molar solutions were made into the tail veins of eighty mice weighing between 19 and 29 grams with an average weight of 24 grams. Twenty mice were used for each compound. In these experiments the injections were made at a constant rate of 6 cc./minute and continued until the animals died. The individual lethal doses in millimoles per gram of each of the citrate solutions injected were averaged for each twenty animals, and the standard deviations determined.

Under ether anesthesia lumbar cordotomies were performed on seven dogs weighing between 10 and 15 kgm. As soon as the animals recovered from the anesthetic the left femoral artery was cannulated and connected to a mercury manometer using heparin as the anticoagulant. Four animals received trisodium citrate (2.94 grams/kilogram/100 cc.) and the other three were given equal molar quantities of citric acid (2.10 grams/kilogram/100 cc.) intravenously at a constant rate of 0.67 cc./minute until death resulted.

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Results. The visible responses of mice, rats, rabbits and dogs to toxic doses of citric acid, monosodium citrate, disodium citrate, and trisodium citrate were similar and consisted primarily of increased general activity, hyperpnea, vasodilatation of the peripheral vessels, salivation, muscle twitching, clonic and tonic convulsions, cyanosis, Cheyne-Stokes respiration and some deaths. In all of the animals receiving single injections except those injected intraperitoneally with citric acid, if recovery occurred it was apparently complete within a few minutes. These findings are explained adequately by the fact that there is formation of double salts with calcium which do not liberate calcium ions (4).

Since this is intended as a comparative study the doses are expressed in millimoles/kilogram in table I instead of the usual grams/kilogram. Because the molecular concentration of the citrate ion was kept constant, in so far as citrate ion effect is concerned, the LD_{50} determinations of the various salts can be compared directly. Citric acid and its sodium salts have the same toxicity when given slowly intravenously to rabbits (see table I). When citric acid was given

TABLE I

The toxicity of citric acid and its sodium salts in millimoles/kilogram as measured by means of the LD_{50}

ANIMAL	NO.	ROUTE OF ADMINISTRATION	CITRIC ACID	MONO-SODIUM CITRATE	DI-SODIUM CITRATE	TRI-SODIUM CITRATE
Rabbits	158	Intravenous at rate of 0.358 millimoles/minute	1.72	1.76	1.77	1.74
White mice	361	Rapid intravenous	0.22	0.23	0.30	0.66
White mice	599	Intraperitoneally	5.0	7.6	7.5	5.5
Albino rats	384	Intraperitoneally	4.6	6.3	7.3	6.0

intraperitoneally to rats and mice a number of animals died as long as one week after recovery from the immediate toxic effects. Gross post-mortem examinations performed upon many of these animals did not reveal the cause of death. If these compounds are injected rapidly intravenously in mice significant differences in the toxicity are observed (see table I). It would appear that the acid rather than the citrate part of the molecule is the cause of this difference in toxicity.

In those experiments on mice in which intravenous injections of 0.25 Molar solutions were made at a constant rate of six cubic centimeters per minute (1.5 millimoles of the drug per minute) no significant differences in the averages of the individual lethal doses were noted. In the 80 experiments (20 for citric acid and 20 for each of its sodium salts) the average dose necessary to kill all of the animals with the standard deviation was 2.08 ± 0.11, 2.01 ± 0.09, 2.21 ± 0.10, and 2.24 ± 0.51 for citric acid, monosodium citrate, disodium citrate and trisodium citrate respectively.

In the experiments on dogs in which blood pressures were recorded there was a gradual fall in blood pressure during citric acid injection until near death when the pressure fell precipitously to zero. When sodium citrate was used the blood

pressure remained fairly normal during the injection until just prior to the death of the animal when it fell abruptly to zero.

Discussion. There are four criticisms to drawing any conclusions as to the effect of citrated blood for transfusion purposes in man from our results: first, our results were obtained from animals and may not be directly applicable to man on a kgm./kgm. basis; second, these animals were injected with pure drug, whereas, the ctirate solution may be altered by the presence of the blood for transfusion; third, the rate of injection in these animals was much more rapid in millimoles/kgm./minute than would ever be given to man in the form of citrated blood; fourth, the patients are not well at the time of transfusion.

Theoretically, ignoring the above criticisms and assuming the LD_{50} for man to be about 1.75 millimoles/kgm., or near that for the slow intravenous injection of rabbits, about one liter of anticoagulant acid citrate dextrose solution U.S.P. would have to be given to a 70 kgm. man in a 15 minute interval or less in order to cause the demise of one-half of the patients so treated.

SUMMARY

1. The train of symptoms following the administration of citric acid and its sodium salts in toxic quantities appears to be identical with that of calcium ion deficiency consisting of increased general activity, hyperpnea, vaso-dilatation of the peripheral vessels, salivation, muscle twitching, clonic and tonic convulsions, cyanosis, Cheyne-Stokes respirations and some deaths.

2. The LD_{50} for citric acid, monosodium citrate, disodium citrate and trisodium citrate in millimoles/kgm. was as follows: intravenous administration in rabbits 1.72, 1.76, 1.77, and 1.74 respectively; for intravenous administration in mice 0.22, 0.23, 0.30, and 0.66 respectively; for intraperitoneal administration in mice 5.0, 7.6, 7.5 and 5.5 respectively; and for intraperitoneal administration in rats 4.6, 6.3, 7.3, and 6.0 respectively. These results suggest that citrate intoxication in massive transfusions does not occur.

We wish to thank Dr. Charles M. Gruber and Dr. Harold W. Jones for their assistance.

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Edwin George Hastings

perimental Biology and Medicine, the Wisconsin Livestock Sanitary Board, the United States Livestock Sanitary Association, and the International Livestock Sanitary Association and was a fellow of the American Association for the Advancement of Science.

It is impossible to estimate the profound influence of E. G. Hastings as a teacher, adviser, and friend upon his students and associates. Evidence of this influence may be found in the long list of well known bacteriologists who worked with him or studied under his direction. It was a memorable experience to know him as "the chief" and to rely upon him for counsel, advice, and stimulation. All who were privileged to know him loved and respected him, and now mourn his death.

W. C. FRAZIER

THE USE OF METABOLITES IN THE RESTORATION OF THE VIABILITY OF HEAT AND CHEMICALLY INACTIVATED ESCHERICHIA COLI[1]

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On the basis of accepted methods for testing sterility, organisms failing to grow in a complete growth medium are considered to be nonviable. It has been observed, however, that bacterial suspensions sterilized by the action of ultraviolet irradiation can be rendered viable when incubated with suitable metabolites (Heinmets, 1953; Heinmets *et al.*, 1954). This suggested that the concept of the "killed" state is relative. In current view the "killed" state would represent only a loss of cellular viability in terms of inability to multiply, but not necessarily in the termination of synthetic and metabolic activity. It appears that the loss of a particular cellular function need not produce the total loss of viability. To the contrary, it may be assumed that when the more basic functional activities of the cellular processes are left essentially intact, the cells may regain viability if "guided" to function specifically to reestablish the specific synthetic capacity necessary for cellular division. This concept presupposes that the synthetic and metabolic processes are more basic in cellular activity than the process of cellular division. Therefore, being only a derivative, fission results from a properly organized and balanced action of basic functional processes. This view is supported by the accepted fact that microorganisms which have lost their ability to divide are still capable of growth. The result is a formation of filamentous variants. Such loss of ability to multiply may be caused by many factors such as: irradiation injury, deficiency of the growth medium, or the presence of certain inhibitors in the medium. All of the aforementioned factors can distort or unbalance the synthetic and metabolic processes of the cell, thus resulting in the impairment of division mechanism.

[1] This work is not to be construed as necessarily reflecting the views of the Department of the Navy.

We have analyzed previously the character of cellular injury produced by ultraviolet irradiation, and in view of experimental evidence a multiple pattern of injury is indicated (Heinmets, 1953). The fact that bacteria can recover from irradiation injury which involves large numbers of structural elements suggested the possibility that when loss of viability is produced by other modes of cellular injury, restoration could take place when suitable conditions are made available. As a result of these considerations, it was decided to investigate the phenomenon of restoration of viability in *Escherichia coli*, strain B/r, when "killed" by the action of heat and chemicals.

EXPERIMENTAL METHODS

Escherichia coli, strain B/r, was used in all experiments. Cultures were grown for 18 hours under constant aeration at 37 C in M-9 medium (Anderson, 1946). The test organisms were removed from the liquid culture media by centrifugation. They were washed twice with distilled water and resuspended in 0.1 M phosphate buffer (pH 7.0). Buffer was composed of KH_2PO_4 and Na_2HPO_4. The number of viable cells in untreated and treated suspensions was determined by plating the bacteria on M-9 agar and incubating for 48 hours at 37 C.

Methods of inactivation. (1) *Heat.* Phosphate buffer, pH 7, was heated to the desired temperature, and one ml of the standard bacterial suspension was added to 99 ml of hot buffer. The mixture was agitated continuously by a magnetic stirrer. Samples were taken at desired time intervals and immediately diluted in a 1:1 ratio with cold buffer. (2) *Chlorine.* To the 99 ml of phosphate buffer which contained one ppm free chlorine was added one ml of the standard bacterial suspension. The mixture was agitated continuously by a magnetic stirrer. Samples

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were removed at desired time intervals, and in order to stop chlorine action the suspension was diluted in 1:1 ratio with a buffer which contained 40 ppm of sodium thiosulfate. (3) *Hydrogen peroxide*, *"zephiran" chloride*, and *ethyl alcohol* also were used for inactivation, and similar procedures as described for chlorine were used. H_2O_2 was neutralized by catalase; and "zephiran" chloride, by a mixture of 3 per cent "tween-80" and 0.2 per cent asolectin. Ethyl alcohol was made ineffective by initial 1:100 dilution of the bacterial suspension with buffer, and the residue of alcohol was removed by dialysis at 5 C. Alcohol also can be removed by washing the bacteria twice by centrifugation.

Methods of reactivation. Bacteria in phosphate buffer (pH 7), after chemical or heat treatment, were diluted in 1:1 ratio with various metabolites (0.2 per cent) or substrates and were incubated for 24 hours at 37 C. Subsequently, all samples were plated out in the usual manner, and the number of viable cells was compared with that of the control suspensions.

RESULTS

Influence of contact time. The effect of the contact time of chlorine on bacterial viability is presented in table 1. It is evident that after one minute of contact with chlorine bacterial suspension did not contain viable cells. This, according to the conventional testing, is interpreted that no cells capable of growing in normal culture medium survived after such treatment. The increase of contact time should potentially increase the probability of total "kill". From a practical point of view, such bacterial suspensions are considered to be sterile. However, when such "sterile" suspensions are incubated for 24 hours in buffered pyruvate solution and subsequently plated, a large number of colonies will develop on M-9 agar plates. The sterile sample (chlorine contact time one minute) contains 2.9 × 10 viable organisms after pyruvate incubation, indicating that chlorine treated samples are "sterile" only when conventional plating procedures are used. Buffer incubation alone did not produce viable cells. Chlorine contact time in this experiment has no significant effect on metabolic recovery. It is likely that bacteria had interacted in a relatively short time with all available chlorine, and further contact time did not have a significant influence on viability.

TABLE 1

Chlorine inactivation and metabolic reactivation of Escherichia coli, strain B/r

Bacteria suspended in phosphate buffer at pH 7, which contains 1 ppm chlorine and samples removed at various time intervals and diluted 1:1 with a solution containing 40 ppm thiosulfate, part of the samples incubated at 37 C for 24 hours and plated.

ORDER NO.	CONTACT TIME WITH CHLORINE	PLATED IMMEDIATELY	INCUBATED IN	
			Pyruvate (0.1%) 24 hours 37 C	Buffer (pH 7)
	min			
1	Control	7.1×10^6	3.3×10^6	—
2	1	0	2.9×10^5	—
3	2	0	2.6×10^5	0
4	4	0	2.5×10^5	—
5	10	0	2.0×10^5	0
6	15	0	1.5×10^5	—

Table 2 summarizes the experiments concerning the heat contact time and subsequent metabolic reactivation. Since ultraviolet inactivation and reactivation experiments had indicated that many other intermediary metabolites besides pyruvate were capable of inducing cellular recovery from radiation injury, it was decided to test other metabolites closely related to pyruvate metabolism. Selection of test temperatures was motivated by the following reasoning. At 60 C for a short period of time, protein denaturation and enzyme inactivation are relatively minor the thermal injury at this level may be partially reversible. On the other hand, at 72 C a more drastic denaturation of proteins and inactivation of enzymes take place and extensive thermal injury is expected. The flash pasteurization method consists of heating liquids to 71.1 C for 15 seconds. Restoration of cellular viability after such treatment was considered highly improbable. The data in table 2 indicate that heat treatment at 60 C for 10 minutes is not sufficient to sterilize the suspension. However, 45 seconds at 72 C were sufficient to sterilize the suspension when plated immediately. Incubation in phosphate buffer at pH 7 did not produce any viable cells in the 45 second sample, whereas the organisms in the 15 second sample lost their viability during incubation. It appears that incubation after heat treatment is detrimental to the bacteria. However, when incubation takes place

TABLE 2

Heat inactivation and metabolic reactivation of Escherichia coli, strain B/r

One ml suspension of bacteria added to 100 ml phosphate buffer at 60 and 71 C; samples removed at suitable intervals and mixed with equal amount of cold buffer; part of the samples incubated at 37 C for 24 hours and plated.

NO.	TYPE OF SAMPLE	VIABLE CELL COUNT			
		1 min at 60 C	10 min at 60 C	15 sec at 72 C	45 sec at 72 C
1	Heated	9.3×10^3	8×10^1	1×10^1	0
2	Heated, incubated in buffer	7.2×10^3	7×10^1	0	0
	Incubated at 37 C for 24 hours with substrate				
3	Acetate	5.5×10^3	4.8×10^3	—	—
4	Oxalacetic acid	3.2×10^6	1.3×10^6	7.2×10^3	4.4×10^3
5	Pyruvate	1.1×10^6	6.0×10^5	1.0×10^3	1.0×10^3

Number of viable cells before heating: (1) Sample heated at 60 C—7.7×10^6; (2) Sample heated at 72 C—1.6×10^6.

in the presence of oxalacetic acid or pyruvate, a substantial increase of viable cells is noted. It is remarkable that bacterial suspension heated at 72 C for 45 seconds exceeding normal pasteurization limits still contains a number of viable cells after incubation with metabolites. Sodium acetate is capable of inducing only minor cellular recovery. It is interesting to note the difference between the organisms' recovery capacity in the presence of the metabolites in regard to heat sensitivity. One minute heat treatment at 60 C indicates a tenfold difference in pyruvate and oxalacetate recovery effect. However, 10 minutes at 60 C destroy all pyruvate recovery but only slightly affect oxalacetate and acetate recovery.

Influence of various metabolites and their combinations on restoration of viability of "killed" bacteria. The original observation that ultraviolet irradiated bacteria can be reactivated following pyruvate incubation (Heinmets, 1953) demonstrated the basic cellular potentiality to overcome, under controlled conditions, a specific mode of injury. It is obvious that in a multiple-pattern injury a broader spectrum of metabolites may induce a more complete cellular recovery. Furthermore, the character of cellular injury will vary, depending on the mode of action of a physical or chemical agent. The chemical mode of injury could be highly specific and could be restricted only to a certain structural element of the cell. Such considerations suggested studies on the reactivating action of various metabolites and their combinations on various modes of cellular injury. Present studies include only a limited number of compounds which are involved in the intermediary metabolism of the cell. In order to rule out multiplication when injured cells are incubated with metabolites which could act as growth stimulants (Johnson and Cohn, 1952), heat or chemically "sterilized" suspensions were plated on M-9 agar or inoculated into nutrient broth to check sterility. No viability could be detected by these methods. A presentation of the effectiveness of various metabolites on heat sterilized bacteria is given in table 3. The original suspension contained 3.1×10^6 viable cells which were heat treated for 30 seconds at 71 C. Incubation for 24 hours in nutrient broth or plating for 48 hours on M-9 agar plates indicated that no viable cells were present. However, incubation of such "sterile" suspensions for 24 hours with various buffered (pH 7) metabolites produces a large number of viable cells. The most active single metabolites are sodium citrate, oxalacetic acid, and lactic acid. A very extensive cellular recovery was achieved when all metabolites were mixed in equal proportions. It is indicated that about 70 per cent of "killed" bacteria have recovered and are capable of growing on M-9 plating medium. Spectroscopic studies indicate that there is no measurable difference in optical density values of bacterial suspensions before and after incubation with a metabolite. This confirms that the presence of viable cells is not due to cellular multiplication.

The observations on the reversal of heat in-

TABLE 3

Restoration of viability by the use of metabolites in Escherichia coli, strain B/r, which have been "killed" by chemicals and heat

SUBSTRATE NUMBER	MODE OF TREATMENT	NUMBER OF VIABLE CELLS				
		Heat	Chlorine	H_2O_2	"Zephiran" chloride	Ethyl alcohol
	Before treatment	3.1×10^6	1.1×10^6	1.4×10^7	3.5×10^6	3.5×10^6
	After treatment	0	0	8.0×10^1	0	0
	Substrate	Treated samples incubated with substrate at 37 C for 24 hours				
1	Buffer (pH 7)	0	0	0	0	3.1×10^3
2	Sodium pyruvate	0	0	8.0×10^1	0	4.4×10^4
3	Oxalacetic acid	2.3×10^2	3.2×10^3	3.0×10^3	1.4×10^2	2.2×10^6
4	Sodium acetate	0	0	1.6×10^2	0	3.6×10^4
5	Sodium citrate	1.3×10^4	1.1×10^4	1.4×10^5	$>1.5 \times 10^6$	4.4×10^1
6	*cis*-Aconitic acid	4.0×10^1	0	—	1.5×10^5	$>1.5 \times 10^6$
7	Isocitric acid	0	0	—	2.8×10^5	3.1×10^4
8	Lactic acid	6.4×10^3	3.4×10^3	0	$>1.5 \times 10^6$	4.4×10^1
9	Malic acid	8.0×10^1	1.5×10^4	5.8×10^2	3.6×10^2	5.0×10^1
10	Succinic acid	1.4×10^2	0	0	0	1.4×10^5
11	α-Ketoglutaric acid	2.1×10^2	0	5.1×10^2	0	$>1.5 \times 10^6$
12	Sodium fumarate	4.1×10^1	0	0	0	2.2×10^4
13	Mixture of: 2, 3, 4	1.0×10^2	0	—	2.5×10^1	5.1×10^4
14	Mixture of: 3, 4, 6	0	2.4×10^4	—	6.0×10^3	2.8×10^4
15	Mixture of: 7, 10, 11	0	0	—	0	1.1×10^5
16	Mixture of: 2 to 12	2.1×10^6	2.5×10^5	1.1×10^6	$>1.5 \times 10^6$	$>1.5 \times 10^6$
17	Nutrient broth	0	0	—	0	—

Contact time of bacteria with heat and various chemicals: (1) heat: 30 seconds at 71 C; (2) chlorine: (1 ppm) 6 minutes; (3) hydrogen peroxide: (0.3%) 25 minutes; (4) ethyl alcohol: (20%) 10 minutes; (5) "zephiran" chloride: (1:1 × 10^4) 6 minutes.

jury by incubation with metabolites suggested studies regarding the injuries produced by various chemicals. From the practical point of view, the chlorine studies are most interesting and important since a major part of the water consumed by civilized populations is sterilized by chlorine treatment. Other chemicals of practical importance which were investigated were hydrogen peroxide, zephiran chloride, and ethyl alcohol. Data concerning these studies are summarized in table 3. Chlorine "sterilized" bacterial suspensions contain viable cells when incubated with sodium citrate, oxalacetic, lactic, and malic acids. The highest number of viable cells is obtained when incubation of "sterile" suspensions takes place in a mixture containing all listed metabolites, but no viable cells develop when incubation takes place in buffer or in nutrient broth. Many of the metabolites which were effective in producing recovery from heat injury are quite ineffective in eliminating the effect of chlorine action. It appears that chlorine action is more specific than heat action which is in general agreement with results observed in protein and enzyme chemistry. Hydrogen peroxide in 0.3 per cent concentration did not produce complete sterility after 20 minutes' contact time with bacteria. However, 24 hours' incubation with buffer sterilized the suspension suggesting that catalase did not decompose all of the hydrogen peroxide. Here also several metabolites are effective in producing cellular recovery; however, the mixture containing all metabolites is again most effective.

Zephiran chloride action on bacteria seems to be reversed very effectively by many metabolites. This suggests that the basic injury produced by zephiran chloride is relatively slight; however, suspensions when incubated in buffer or in nutrient broth remain sterile.

Ethyl alcohol concentration was kept relatively low (20 per cent) in order to avoid excessive

cellular injury. After ten minutes' contact time with alcohol, bacterial suspension was diluted in 1:10 ratio with buffer and one part plated immediately and the other incubated for 24 hours with metabolites. Under these conditions no viable cells were observed in any of the incubated samples. It was evident that residual alcohol (2 per cent) was capable of suppressing the cellular recovery. Earlier experiments had indicated that at such concentration ethyl alcohol did not have any significant effect on normal bacteria. Experiments were repeated, and after 20 minutes' contact time bacteria-alcohol mixture was diluted in 1:10 ratio with cold buffer (5 C) and dialyzed under sterile conditions. A small agitating type of electrophoresis dialyzer (Aminco) was used. Buffer was replaced three times after two hours of each dialyzing cycle. Bacteria were incubated subsequently in metabolites and plated. It is evident that buffer incubation alone is capable of restoring some number of viable cells, but addition of metabolites produces a broad spectrum of recovery and in some cases probably complete restoration of viability. This suggests that the action of alcohol at such concentrations produces only a very superficial cellular injury. In other experiments, where contact time was increased even to 25 minutes, a number of viable cells was produced when a sterile suspension was incubated with oxalacetic acid.

Effect of metabolites when present in agar M-9 plates. It was desirable to determine whether the addition of metabolites to the plating medium would induce cellular recovery. One part of sterile bacterial suspension (sterilized by various methods) was plated on supplemented, M-9 agar (table 3, mixture 16) and M-9 agar alone. The other part of the sample was incubated 24 hours in buffered metabolites (table 3, mixture 16) and plated out on M-9 agar. An increase of viable cell count or loss of sterility was observed only on occasions when treated bacterial suspensions were preincubated with metabolites, indicating that the presence of metabolites in agar plating medium is not inducive to cellular recovery. However, when bacteria after incubation with metabolites are plated on M-9 medium, which also contains metabolites, an increased number of colonies will develop. This phenomenon suggests that some bacteria during the incubation have adapted to the substrate as carbon source and will develop normal colonies when this substrate is present in the plating medium. Further studies are desired on this subject since restoration of cellular viability by adaptation to a new carbon source may be helpful for elucidating the mechanism of the reactivation process.

How is growth controlled or prevented when the "killed" bacteria are incubated with metabolite? In sterility experiments, bacterial samples after treatment with chemicals or heat were tested in several ways for viable cells: A large inoculum (1 ml, containing 1 × 10^6 cells) of treated suspension was used to seed nutrient broth and incubated for 24 hours. When no visible growth developed, the suspension in nutrient broth was tested for growth by plating 0.1 ml samples on M-9 agar. When no colonies appeared after 48 hours of incubation, the suspension was considered sterile. Further tests consisted of plating bacterial suspensions immediately after treatment with chemicals or heat and after incubation of samples in buffer for 24 hours at 37 C. Phosphate buffer (pH 7.0) is not toxic to partially inactivated cells as indicated by other numerous experiments (Heinmets *et al., in press*).

When all described tests did not produce viable cells, the suspension was considered to be sterile. It is obvious that when such sterile bacterial suspension is incubated with metabolites and viable cells appear, as indicated by plating, at least the first generation of cells has to recover before multiplication can take place. What about subsequent multiplication after the recovery? It is obvious that an increase of optical density has to take place when a large number of additional cells develop in bacterial suspension (for example: 50 per cent of original count). However, measurements do not indicate any increase of optical density. One of the possibilities might be that some disintegration of bacteria might take place when suspension is incubated with metabolites. This is contradicted by experimental evidence that optical density is constant when normal cells are incubated in metabolites or treated cells are incubated in buffer. Such data suggest that no significant multiplication will take place in bacterial suspensions while incubated with metabolites. However, there is direct evidence that bacterial growth and reactivation processes are different types of phenomena and reactivation can take place in conditions where growth is impossible. The fol-

lowing example will serve to illustrate the issue. When a bacterial suspension containing 7.8 log units of viable cells is irradiated with ultraviolet (a dose that reduces the viable log count to 3.7) and then is incubated with 0.01 M oxalacetate in the presence of 0.2 M sodium fluoride, a two log unit increase of viable cell count takes place. However, sodium fluoride at such a concentration is inhibitory to the growth.

In summary, when injured bacteria are incubated with metabolites, the increase of viable cell count is produced essentially by reactivation but not by multiplication.

DISCUSSION

Knox *et al.* (1948) working with *E. coli* demonstrated that chlorine in bactericidal concentrations reduced the activity of various sulfhydryl enzymes and other enzymes sensitive to oxidation. The action of quaternary ammonium compounds is not entirely clear, but complex formation between the components of the cell wall and surface active agent seems to be the primary step in the inactivation process (Lawrence, 1950). Heat, hydrogen peroxide, and alcohol are all capable of inactivating enzymes, but most of the work has been performed on isolated enzyme preparations. Relatively few studies have been performed with the enzyme systems of the entire cell which have been subjected to various modes of injury. Oliver (1952) found that the activity of the decarboxylating enzymes of *E. coli* ceased above 80 C but recommenced on a return to lower temperatures. He also observed that *E. coli* suspensions killed by heat and chemicals were still sterile after 7 days' incubation in culture medium but possessed almost normal decarboxylase activity which indicated that loss of viability does not represent the loss of all enzyme activities. It is obvious that some other enzymes may have been inactivated by heat action. For example, when *E. coli* are inactivated by ultraviolet irradiation, the ability to oxidize glucose is only slightly affected, but pyruvate oxidation is suppressed extensively (to be published). Our measurements indicate that over a million hypochloride molecules or ions are required to "kill" one organism (*E. coli*, strain B/r). Since chlorine reacts with cellular sites irreversibly, this suggests that a large number of enzymes are inactivated in the process of killing one bacterium. Calculations on the uptake of the other bactericidal agents used in these experiments indicate that a large number of molecules interact before bacterial inactivation results. An indication of the diversity and severity of cellular injury can be seen when data in table 3 are compared. Zephiran chloride and ethyl alcohol produce a less severe injury than chlorine.

The phenomenon that cells fail to grow or multiply in normal culture medium after subjection to chemical or heat injury but still exhibit some metabolic and synthetic activity suggests that the normal nutritional pathway(s) has become inoperative. However, under such conditions, the cell still could be functioning partially in processes involved in intermediary metabolism. It was speculated that, if a route of entry into respiratory cycle(s) could be found with a proper metabolite, a partial restoration of cyclic and interrelated processes could take place. Such a phenomenon could be visualized as a stepwise operation in a chain process where metabolite induces the enzyme formation and subsequent enzymatic conversion of metabolite would provide a new metabolite for other enzyme formation. In such a manner, a complete cycle could become operative. Such mechanism would be similar in principle to simultaneous enzyme adaptation as proposed by Stanier (1947).

If an extensive block would exist in one of the steps in the cycle, the inherent character of feedback mechanism of cyclic processes would permit an alternate route through a pathway in the reverse direction. Rate of such recovery seems to have an asymptotic characteristic. Obviously the possibility exists that a recovered cell may adapt itself to the original metabolite as carbon source. That such a phenomenon takes place is indicated by experimental evidence that the presence of the metabolite in plating medium produces higher rate of the reactivation. However, the fact that large numbers of cells are capable of growing nutrient agar plates containing only glucose as carbon source suggests that normal nutritional pathways again may have become operative during the process of reactivation. This suggests that pathways of glucose metabolism also could become operative during the establishment of cyclic processes.

What is the evidence for the existence of respiratory cycle(s) in bacteria *E. coli*, and what influence does the abnormal functioning of the cycle(s) have on the division process of bacteria

There is only a limited amount of data available in the literature on this subject. Umbreit's (1953) analysis suggests that citric acid cycle and possibly some other cycles are operative in *E. coli*. Nickerson and Sherman (1952) have shown that undividing filamentous bacteria (*Bacillus cereus*) produced by magnesium deficiency had much lower pyruvate oxidation rate than normally dividing cells. In view of such evidence, lowered or blocked reaction processes in citric acid cycle may lead to disability of the cell to divide; restoration of normal cyclic processes may help to restore cellular viability. New enzyme formation could be a part of such reactivation mechanism since there is enough evidence that cells rendered nonviable are capable of forming enzymes (Stephenson and Yudkin, 1936; Baron *et al.*, 1953). Since our experimental data reveal that metabolites belonging to the citric acid cycle were effective in restoring cellular viability, metabolic reactivation may be operative on the principles of cyclic processes. However, other modes of recovery may be possible, depending on the character of the injury pattern.

An alternative mode of action of metabolites in the reactivation of "killed" bacteria could be interpreted as providing a supply of more readily available energy. Johnson and Cohn (1952) studied the effect of various tricarboxylic acids on *E. coli*, strain 221, and found that in a one per cent glucose medium, malate, oxalacetate, fumarate, and citrate considerably increased the growth rate. Their findings indicate that the growth of *E. coli* in a glucose medium is limited by the inability of Kreb's cycle enzymatic system or a part of this system to provide some of the components of the tricarboxylic acid cycle in rates adequate for maximum growth. Tricarboxylic acids would serve as immediately utilizable compounds as energy sources.

If "killed" bacteria fail to grow because energy is not readily available, then the addition of suitable metabolites to the plating medium should provide substrates which are immediately utilizable as energy sources. Experiments indicate that no significant reactivation takes place under these conditions. Addition of metabolites to the liquid nutrient growth medium does produce viable cells, but here it is impossible to estimate the rate of recovery since recovery and growth processes are superimposed. Incubation of "killed" bacteria with metabolites for one or two hours prior to the plating does not produce viable cells or increase the viable cell count. Since this time is sufficiently long to establish the equilibrium between the cell and metabolite, it appears that longer contact time is essential for other reasons.

There are some practical aspects of the metabolic recovery phenomenon which deserve attention. At present the sterility of a medium is tested by methods involving culture plate or culture tube techniques. Table 3 indicates that treated bacterial suspensions which are shown to be sterile by direct plating or broth methods yield a large number of viable cells when incubated with metabolites. It is obvious that present concepts of sterility are entirely arbitrary. The metabolic reactivation phenomenon indicates that a particular medium tested for sterility by conventional methods may be rendered septic when it comes into contact under suitable conditions with known or unknown metabolites; i.e., vaccines and other sterile supplies. This indicates that sterility standards may have to be raised to levels where metabolic recovery also fails in order to secure complete sterility.

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SUMMARY

Experiments indicate that suspensions of *Escherichia coli*, strain B/r, which have been sterilized by the action of heat, chlorine, "zephiran" chloride, and ethyl alcohol contain viable cells when incubated with various metabolites of the tricarboxylic acid cycle. When such "sterile" suspensions are incubated in buffer or in nutrient broth, no viable cells can be demonstrated. In the present series, the following metabolites were most effective in producing reactivation: (1) Heat "killed": Sodium citrate, lactic acid, and oxalacetic acid; (2) Chlorine "killed": Sodium citrate, malic acid, and oxalacetic acid; (3) Hydrogen peroxide "killed": Sodium citrate, lactic acid, *cis*-aconitic acid; (4) Zephiran chloride "killed": Sodium citrate, lactic acid, *cis*-aconitic acid, and isocitric acid; (5) Ethyl alcohol "killed": *cis*-Aconitic acid, α-ketoglutaric acid, succinic acid, etc; (6) The combination of 11 metabolites produced the highest reactivation.

Reactivation processes of bacterial cells are discussed in terms of resynthesis of enzymes and reestablishment of cyclic processes.

It is indicated that conventional testing and culturing methods are not adequate to determine the levels of "complete" sterility.

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THE MORPHOLOGY, CELL STRUCTURE, AND TAXONOMIC AFFINITIES OF THE MORAXELLA

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Difficulties were encountered in the identification of a gram negative, encapsulated coccobacillus (strain 1297) that produced oxidase positive mucoid colonies. Final identification as a mucoid variant of *Moraxella non liquefaciens* was not achieved until Henriksen's helpful paper was published (1952) and strains were exchanged with him. The peculiarities of structure and arrangement, as well as the favorably large cells, demanded a comparative study of the species of *Moraxella* and of organisms that may be related to that genus.

Lwoff (1939) proposed the creation of a new genus, *Moraxella*, to include the organisms classified at that time as *Hemophilus duplex* and also some apparent relatives. He suggested that this genus should be separated from the *Hemophileae* because the organisms were morphologically dissimilar and did not require hematin or phosphopyridine nucleotide as growth factors. These proposals were supported and extended by Audureau (1940). Although the new genus was accepted in *Bergey's Manual* (Breed *et al.*, 1948), it was retained in the tribe *Hemophileae*. We have included a morphological comparison with *Hemophilus* species because of these classificatory associations and because strain 1297 (but none of the others) showed some requirement for hematin on primary isolation. Consideration is given also to Henriksen's opinion (1952) that *Moraxella* and *Neisseria* share many characteristics.

MATERIALS AND METHODS

Strains studied. M. non liquefaciens (mucoid strains): 3452 (see Henriksen, 1951, 1952), 1297 (isolated from throat swab in this laboratory in 1950), and 87 (isolated from feces in 1953 by Dr. F. W. Jelks).

M. non liquefaciens (nonmucoid strains)-18522/51, 564/51, 29128/52, 30412, 11865/51, 1890/51, 752/52, 361/52, OT/51, 19116/52 (all obtained from Dr. S. D. Henriksen), and 580 (isolated from throat swab in this laboratory in 1953).

Some authentic strains of *Moraxella* were obtained from Dr. Henriksen who had received them from the Institut Pasteur, Paris. These included: *M. lacunata* (strains 4238 and "Diplobacillus of Morax, 260"); *M. liquefaciens* ("Diplobacillus of Petit"); and *M. lwoffi* var. *bacteroides* and *M. pericardite*.

Dr. Margaret Pittman provided us with three strains of *M. bovis* and with a number of representative *Hemophilus* species (*H. influenzae*, *H. suis*, *H. aegyptius*, *H. haemolyticus*, *H. parahaemolyticus*, *H. parainfluenzae*, *H. haemoglobinophilus*, *H. piscium*, and *H. pertussis*). A number of strains of *Neisseria* (including *N. meningitidis* and *N. catarrhalis*) were isolated and characterized in this laboratory.

Cultivation. The observations were made on cells grown and maintained on 4 per cent human blood agar (details of a proteose peptone-papain broth base were given by Murray and Pearce, 1949). Special tests mentioned in the text were standard methods described in *The Manual of Methods for Pure Culture of Bacteria*.

General cytological techniques. For the study of cell habit and of cytoplasmic structure cells were fixed *in situ* through the agar with Bouin's fluid (see Klieneberger-Nobel, 1950). These preparations on coverslips were stained lightly with 0.01 per cent thionin and mounted in water.

Nuclear structures were studied most conveniently in impression preparations of cells fixed with osmium tetroxide vapor, which were treated then with N-HCl at 60 C (for times varying from 5 to 12 minutes according to the physiological state of the cell), washed, and stained either with diluted Giemsa (Robinow, 1942) or with 0.2 per cent basic fuchsin (Cassel, 1950). Additional treatment with 5 per cent tannic acid (Cassel, 1952) often allowed clearer differen-

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Bacterial Growth by Shake Culture Method and the Growth Stimulating Effect of Some Organic Acids Related to TCA-cycle.

I. Escherichia Coli and Salmonella Typhi

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(The manuscript was received on March 1, 1954).

Introduction:

The quiet culture method has ordinarily been used for the growth culturing of bacteria; however,this method contains a number of inconveniences for obtaining a large amount of bacteria in a short period of time. As the methods for obtaining a large amount of bacteria, the shake culture method or the aeration culture method have often been used for the growth of aerobic bacteria and "deviating" anaerobic bacteria. The report written by Mihashi et al. seems to be one of the few systematic studies on the shake culture method (1).

It has been becoming increasing clear that, in recent years, a growth stimulating effect is observed when an organic acid related to the TCA-cycle is added. In the case of Escherichia coli, Johnson and Cohn (2,3) showed that the growth would double by the addition of L-malate and fumarate. Caswell et al. (4) discovered the fact that, in the case of vacillus lactis, fumarate and ethylsodium oxalacetate can take the place of tomato juice.

Wieland et al. (5) obtained the maximum growth by adding citrate to fumarate and ethyl sodium oxalacetate. In addition, Skeggs et al. (6) reported that the growth of lactobacillus leishmanii is promoted by malate, citrate and (alpha)-keto-glutarate, etc. In view of the aforementioned, the present author carried out a systematic study on the effect of the shake culture method and the organic acids related to the TCA-cycle upon various bacteria for the purpose of improving the growth of bacteria to a greater extent than by the present culture media

In the present study, it will be shown that a highly satisfactory growth of Escherichia coli is obtained when the shake culture method is used with an

addition of succinate or fumarate and that, when salmonella typhi is subjected to the shake culture method with an addition of succinate or citrate, an optical density as much as approximately seven times that which is obtained in the case of a quiet culture method without using an organic acid is obtained.

Materials Used and Method Employed in the Experiment

Bacteria Used: The Escherichia coli and Salmonella typhi 58 in the storage room of our Department were used.

Culture Media Used: As a base culture medium, 9.0 grams of potassium phosphorous, 0.1 gram of magnesium sulfate, 1.0 gram of ammonium sulfate and 2.0 grams of dextrose (grape sugar) were dissolved in one liter of distilled water to have the pH at 7.0, with high pressure sterilization having been carried out, were used in the case of Escherichia coli. In the case of Salmonella typhi, 1.4 grams of sodium phosphorous, 1.0 gram of potassium phosphorous, 2.0 grams of table salt, 0.1 gram of magnesium sulfate, 2.0 grams of dextrose, 2.0 grams of casamino acids technical Difco, 10 milligrams of thiamine and 25 milligrams of tryptophane were dissolved in one liter of distilled water to have the pH at 7.0, with high-pressure sterilization having been carried out, were used.

In carrying out the experiment, moreover, sodium succinate, sodium fumarate and sodium citrate were added to the base culture medium so as to show M/10, M/50 and M/100 and five milliliters of each was poured into a test tube for use.

Culturing and the Method for the Determination of Results:

The aforementioned bacterial stock, which had been cultured for a period of 20 hours on an ordinary isinglas slant, was scraped, and was cleaned three times with sterilized table salt water. The same was caused to float on sterilized table salt water so that each would become 2.5 milligrams. One platinum ear of the same was inoculated and both the quiet culture and shake culture of the same were carried out at the temperature of 37 degrees centigrade.

The "shake" was carried out at an amplitude of 35 centimeters and a number of shakes amounting to 200 cycles per minute. The extent of the growth of

the bacteria was expressed by optical density by using a photoelectric colorimeter.

Experimental Result

1. Effect of Shake upon Growth:

Both in the case of Escherichia coli and Salmonella typhi, the growth was found to be extremely better in the shake culture method than in the quiet culture method.

Up till approximately the sixth hour of culturing, there was observed almost no difference between the two culture methods. From around the 18th hour, however, approximately twice the optical density was obtained in the shake culture method as compared with the optical density obtained in the quiet culture method.

(Insert Figure 1 on p. 353. Effect of succinate upon the growth of Escherichia coli. a. Culturing time. b. Hour. Note: 1. The solid line indicates shake culture; 2. The dotted line indicates quiet culture; 3. The fine line indicates a medium with the addition of an organic acid; and 4. The thick line indicates a medium without such an addition.)

2. Effect of Organic Acies upon Growth:

(1) Sodium succinate: In the case of Escherichia coli, sodium succinate had the effect of extending the induction period and increasing the speed of the break-up of the bacteria during the logarithmic multiplication period in the quiet culture method and increasing optical density after 48 hours.

In shake culture, there were observed an increase in the rate of the break-up of bacteria and the shortening of the time before the maximum growth period was reached. Accordingly, the growth stimulating effect of succinate was observed to a marked extent. In addition, the growth stimulating effect was in proportion to the density of succinate. (Reference should be made to Figure 1.)

(Insert Figure 2 on p. 353. Effect of succinate upon the growth of Salmonella typhi. a. Culturing time. b. Hour. Notes: Same as in Figure 1.)

Succinate was observed to have a growth stimulating effect on Salmonella typhi both in the quiet culture and shake culture methods. An extension of the induction periods as in the quiet culture method of Escherichia coli was not observed in this case. (Reference should be made to Figure 2.)

(2) Sodium fumarate: Inthe case of Escherichia coli, sodium fumarate had the same effect on the induction period as that which was obtained without the addition of an organic acid. In this case, however,the rate of the breakup of the bacteria becomes slightly higher, the logarithmic multiplication period continues for a longer period of time and there is a tendency for the amount of grown bacterial to become extremely large. The effect of the fumarate was in direct proportion to its density.

In the shake culture method, the induction period was found to be the same as in the case where no organic acid was added. In this case, however, the rate of the break-up of the bacteria was high, the lengthof time before the maximum growth period was reached was shortened and the amount of the grown bacteria was also large. However, this effect does not seem to be closely related to the density of the fumarate.

At M/50, in other words, the effect of the same was the greatest and was almost the same as in the case of M/10. (Reference should be made to Figure 3).

(Insert Figure 3 on p. 354. Effect of fumarate upon the growth of Escherichia coli. a. Culturing time. b. Hour. Notes: The same as in Figure 1.)

(Insert Figure 4 on p. 354. Effect of fumarate upon the growth of Salmonella typhi. a. Culturing time. b. Hour. Notes: same as in Figure 1.)

No significant difference was observed in the case of Salmonella typhi by the addition of fumarate in quiet culture. In the shake culture method, however, an increase in the rate of the break-up of the bacteria and an increase in the amount of grown bacteria were observed at the density of M/10. At M/50 and M/100, where the concentration of fumarate was small, no difference was observed in shake culture as compared with the case where an organic acid was added. Rather, its optical density was found to have dropped slightly

after culturing for a period of 48 hours. (Reference should be made to Figure 4.)

(3) Sodium citrate:

Sodium citrate serves to slightly reduce the growth of Escherichia coli at M/10 in quiet culture. At M/50 and M/100, however,the logarithmic multiplication period was extended and its optical density was found to be greater after 48 hours as compared with the case where no organic acid was added.

The effect was slightly greater at M/50 as compared with M/100. In shake culture, the rate of the break-up of the bacteria dropped at M/10; however, after 48 hours, an optical density which was slightly inferior to the case where no organic acid was added was shown. The rate of the break-up of the bacteria became large at M/50 and M/100; however, the maximum growth period was reached at the 18th hour, with the optical density being reduced thereafter. The optical density in the maximum growth period was observed to be no different from the case where no organic acid was added. (Reference should be made to Figure 5.)

(Insert Figure 5on p. 354. Effect of citrate upon the growth of Escherichia coli. a. Culturing time. b. Hour. Notes; Same as in Figure 1.)

The growth inhibitive phenomenon of citrate at high concentration upon Salmonella typhi was not observed in both quiet culture and shake culture. On the other hand, a marked growth promoting effect alone was observed. In other words, the optical density at the 48th hour in quiet culture was large in proportion to the concentration of the citrate. In the shake culture method, on the other hand, the incline of the logarithm ultiplication curve was sharp and an increase in this break-up rate was observed. In addition, on the 18th hour which was the maximum growth period in the case where no organic acid was added, the optical density was found to be more than three times as high as in the case where there was no addition. (Reference should be made to Figure 6.).

(Insert Figure 6 on p. 355. Effect of citrate upon the growth of Salmonella typhi. a. culturing time. b. Hour. Notes: Same as in Figure 1.)

Observations:

It has been known in the past that the growth of the bacteria is promoted by shake culture in the case of deviating anaerobic bacteria. Relative to the phenomenon of the increased growth of bacteria by shake culture, Mihashi et al. (1) mainly emphasized an increase in the component pressure of oxygen in the culture medium. It is not possible to ignore the increase in the area of a culture medium by shaking.

Heukelekian and Heller (7) admitted the fact that, in the case of Escherichia coli, an increase in the area of the culture medium gives a promoting effect upon the growth of the bacteria and observed that the reason for the above phenomenon lay in the fact that the nutrient substance of a positive electric charge was accumulated on the boundary surface between the culture medium and the solid or between the culture medium and air.

Masui et al. (8) utilized this phenomenon and, in connection with the culturing of Hemophilus pertussis whose culture had been difficult to carry out in the past, they threw glass balls into the culture medium, thereby increasing the area of the culture medium and obtaining a satisfactory result. Accordingly, it may be stated that an increase in the area of the boundary surface between the culture medium and air by shaking gives a satisfactory effect upon the multiplication of the bacteria in shake culture as compared with quiet culture.

According to the experiments which the author carried out, succinate and fumarate serve to stimulate the growth both in shake culture and in quiet culture in the case of Escherichia coli and both succinate and citrate serve to stimulate the growth in the case of Salmonella typhi.

As a factor for the aforementioned phenomenon, it is suspected that the "synthesizing" capability of succinate and fumarate is not exerted to an extent sufficient to carry out full-fledged growth in the case of Escherichia coli and the synthesizing capability of succinate and citrate is not exerted to an extent sufficient to carry out full-fledged growth in the case of Salmon-

ella typhi.

The organic acids which are related to the T.C.A.-cycle are different from glucose and have the capacity to be used directly as a source of force. Since in both bacterial stocks the existence of enzymes related to the TCA-cycle has been proved (9, 10), there is no denying the possibility that succinate, fumarate and citrate are directly utilized as a source of force. In order for these organic acids to be utilized as a source of force, however, the amount of the enzyme in the bacteria and the permeability of the organic acid into the bacteria are believed to be involved. Even when the organic acids are of the same concentration, therefore, it is natural that there is a difference between the two bacterial stocks in the growth stimulating effect.

In addition, citrate rather serves to obstruct the growth of Escherichia coli at high concentration. Therefore, it is indeed interesting to observe a marked growth stimulating effect on Salmonella typhi.

In the case of quiet culture, there is a long period of time before the maximum growth period is reached irrespective of whether an organic acid was added or not added. In the case of shake culture, however, the time involved is generally short and this was markedly observed in the case where an organic acid was added. This is indicative of the fact that the production of a suitable enzyme for a bacterium is delayed in quiet culture. Thus, there is a difference in time necessary for acquiring the source of force.

Conclusion:

By using Escherichia coli and Salmonella typhi, the effect of the shake culture method and sodium succinate, sodium fumarate and sodium citrate amongst the various organic acids related to the TCA cycle upon their growth were examined and the following results were obtained:

1. In shake culture, a growth which is approximately twice in terms of optical density as in quite culture was observed.

2. A marked growth stimulating effect was observed in the case of Escherichia coli by the addition of sodium succinate or sodium fumarate.

3. A marked growth stimulating effect was observed upon Salmonella typhi

by the addition of sodium succinate or sodium citrate.

At the end of this report, the author wishes to express his deep gratitude to Professors Kuwashima and Masui, who guided him constantly and who read his manuscript critically. In addition, he offers sincere thanks to Miss Mitsuko Kubo who extended enthusiastic assistance to him throughout the experiments.

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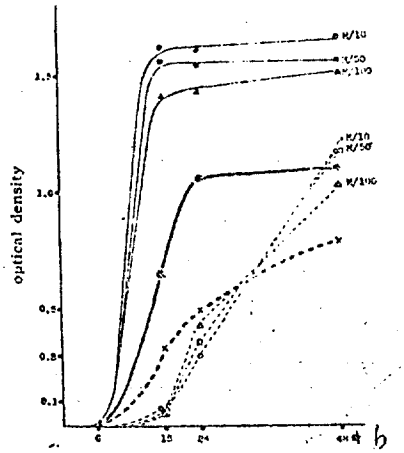

Figure 1

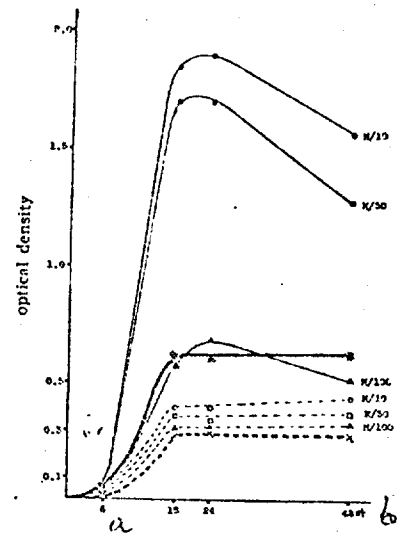

Figure 2

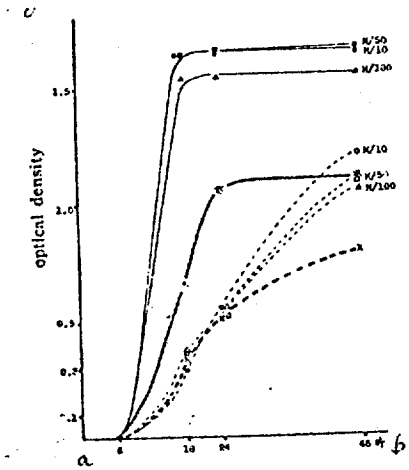

Figure 3

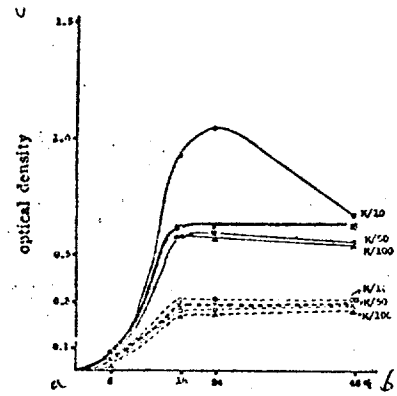

Figure 4

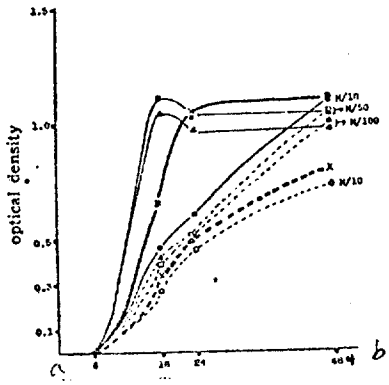

Figure 5

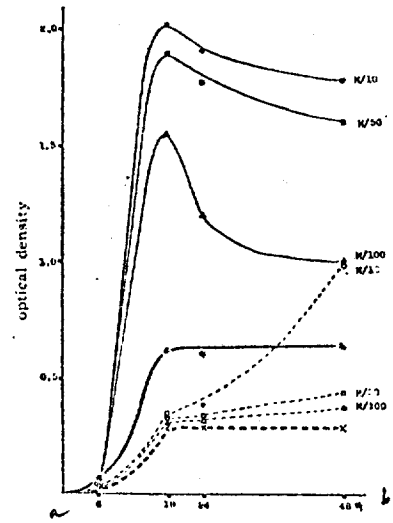

Figure 6

182 Sodium citrate

諸種細菌の振盪培養と T.C.A.-cycle 關與有機酸の發育に及ぼす影響

I Escherichia Coli 及び Salmonella Typhi

Bacterial Growth by Shake Culture Method and the Growth Stimulating Effect of Some Organic Acids Related to T.C.A.-cycle

I. Escherichia Coli and Salmonella Typhi

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(昭和29年3月1日受付)

緒言

細菌の培養は通常静置培養により行われているが多量の菌を短期間に得る為には種々の不便があつた。多量の菌を得る方法としては好気性菌、偏性嫌気性菌に対して振盪培養或は通気培養が屢々行われて居る振盪培養に關する系統的な研究は三橋等[1]の報告が見られるに過ぎない。

又 T.C.A.-cycle 關与有機酸を添加すると発育促進効果が認められることも近来次第に明らかにされつつある。Escherichia coli に対しては Johnson and Cohn[2,3] は L-Malate 及び Fumarate の添加に依りその発育が 2 倍に増加することを示し、Caswell et al[4] は Lactobacillus lactis で Fumarate と Ethylsodium oxalacetate がトマトジュースに代替し得ることを見出し、Wieland et al[5] は Citrate を Fumarate 及び Ethyl sodium oxalacetate に添加して最大の発育を得、又 Skeggs et al[6] は Malate, Citrate, α-ketoglutarate 等によつて Lactobacillus leishmanii の発育が促進されると報告している。筆者は以上の事実に鑑み、細菌の現行の培地よりも発育を良好にする目的で、各種細菌について振盪及び T.C.A.-cycle 關与有機酸の発育に及ぼす影響について系統的研究を行つた。

本報に於ては Escherichia coli が Succinate 或は Fumarate を加えて振盪すると極めて良好に発育すること、及び Salmonella typhi が Succinate 或は Citrate を添加して振盪培養すると有機酸を加えないで静置培養した場合に比して実に約7倍の optical density を示すことを知つたので報告する。

実験材料及び方法

使用菌株： 当教室保存の Escherichia coli 及び Salmonella typhi 58 を使用した。

供試培地： 基礎培地としては Escherichia coli には第1燐酸加里 9.0g, 硫酸マグネシア 0.1g, 硫酸アンモン 1.0g, 蒸溜水 2.0g を蒸溜水 1l. に溶解して pH7.0 として高圧滅菌して用いた。又 Salmonella typhi には第1燐酸ソーダ 1.4g, 第1燐酸加里 1.0g, 食塩 2.0g, 硫酸マグネシア 0.1g, 蒸溜水 2.0g, Casamino acids technical Difco 2.0g, Thiamine 10mg, Tryptophane 25mg を蒸溜水 1l. に溶解し pH7.0 として高圧滅菌して用いた。

而して実験に際しては Sodium succinate, Sodium fumarate 及び Sodium citrate を M/10, M/50 及び M/100 になる如く基礎培地に添加して 5ml. 宛試験管に分注して使用した。

培養及び成績の判定法： 前記菌株の普通寒天斜面 20時間培養菌体を掻取り 3 回減菌生理食塩水で洗滌後各々が 2.5mg/ml になる如く減菌生理食塩水に浮遊させその 1 白金耳を接種し 37°C で静置及び振盪培養を行つた。

振盪は振幅 3.5cm, 振盪回数 200cycle/min で行つた。菌の発育度は光電比濁計を用い optical density であらわした。

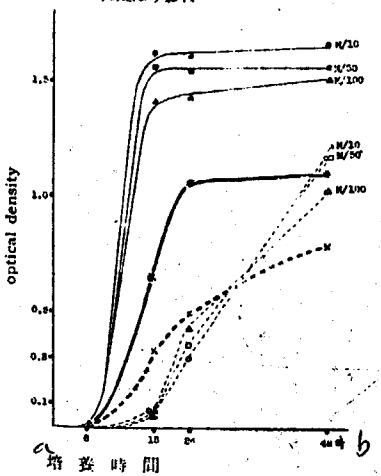
実験成績

1. 振盪の発育に及ぼす影響 Escherichia coli 及び Salmonella typhi 共に振盪培養の方が静置培養に比して極めて発育が旺盛であつた。

培養後 6 時間目位迄は両培養法に差は認められないが、18時間目から振盪培養の方が optical density にして静置培養の約 2 倍という極めて旺盛な発育が認められた。

Fig 1

第1図 Succinate の Escherichia coli の発育に及ぼす影響



註：1. 実線は振盪培養
2. 点線は静置培養
3. 細線は有機酸添加培地
4. 太線は無添加培地

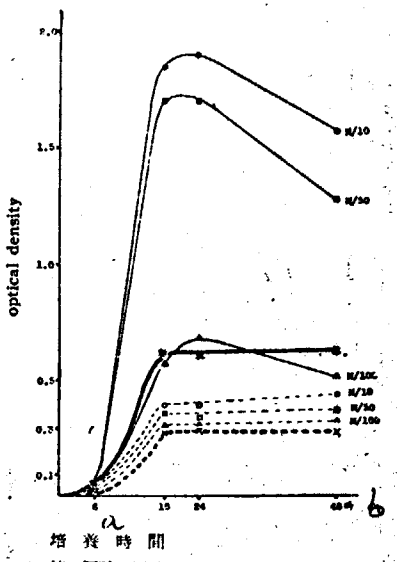
2. 有機酸の発育に及ぼす影響：

(1) Sodium succinate： Sodium succinate は Escherichia coli に対し静置培養では誘導期の延長と対数増殖期の菌の分裂速度の増加及び 48 時間後に於ける optical density の増加が見られた。

振盪培養では菌の分裂速度の増加と最大発育期に至る迄の時間の短縮が見られ、従つて Succinate による発育促進効果が著明に認められた。同発育促進効果

Fig 2

第2図 Succinate の Salmonella typhi の発育に及ぼす影響



註：第1図註に同じ

は Succinate の濃度に比例した（第1図）。

Salmonella typhi に対しては Succinate は静置培養及び振盪培養共に発育促進作用が認められ、Escherichia coli の場合の如く静置培養に於ける誘導期の延長は見られなかつた（第2図）。

(2) Sodium fumarate： Sodium fumarate は Escherichia coli に対し静置培養では誘導期は有機酸無添加の場合と同様であるが、菌の分裂速度が稍々大となり、しかも対数増殖期が長く続き発育距離は極めて大となる傾向が見られた。又 Fumarate の効果はその濃度に比例していた。

振盪培養では誘導期は有機酸無添加の場合と同様で

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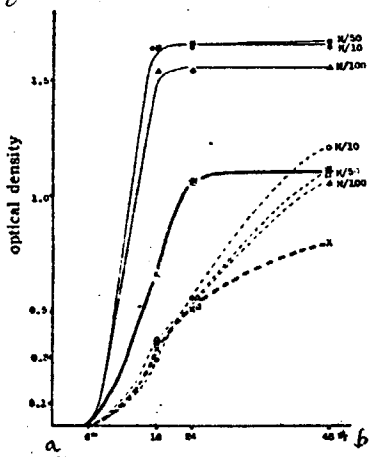
あるが，菌の分裂速度が大で且つ最大発育期に至る迄の時間が短く，しかも発育菌量も大であつた．然し乍ら此の作用は Fumarate の濃度には余り関係が無い様であつた．

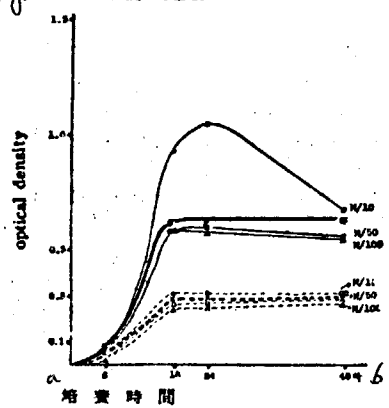
即ち M/50 に於てその効果は最大で M/10 の場合と殆ど差が認められなかつた（第3図）．

Fig 3 第3図　Fumarate の Escherichia coli の発育に及ぼす影響



註：第1図註に同じ

Fig 4 第4図　Fumarate の Salmonella typhi の発育に及ぼす影響



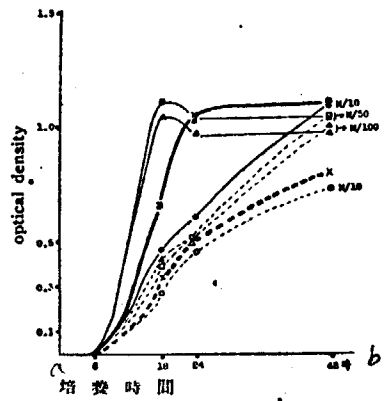
註：第1図註に同じ

Salmonella typhi に対しては静置培養では Fumarate 添加に依り有意な差が認められなかつたが，振盪培養では M/10 の濃度の場合には菌の分裂速度の増大及び発育菌量の増加が認められた．Fumarate の濃度の小なる M/50及びM/100の場合は振盪培養を行つても有機酸無添加の場合と差が認められなかつた．それに48時間培養後にはむしろ optical density が稍々低下した（第4図）．

(3) **Sodium citrate**：Sodium citrate は Escherichia coli に対し静置培養では M/10 の場合には稍々発育阻害的に働くが，M/50 及びM/100 では対数増殖期を延長させ，48時間後には有機酸無添加の場合に比し optical density が大となつた．

しかも M/50 の方が M/100 に比して効果が稍々大であつた．振盪培養の場合は M/10 の濃度では菌の分裂速度を低下させるが，48時間後には有機酸無添加の場合に稍々劣る optical density を示す程度になつた．M/50及び M/100 では菌の分裂速度は大となるが，培養18時間目に最大発育期に達し以後 optical density は減少したが最大発育期の optical density は有機酸無添加の場合と差が認められなかつた（第5図）．

Fig 5 第5図　Citrate の Escherichia coli の発育に及ぼす影響

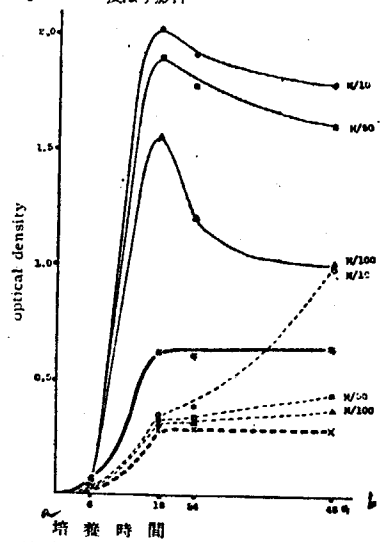


註：第1図註に同じ

又 Salmonella typhi に対しては高濃度の Citrate の発育阻止現象は静置培養及び振盪培養共に認められず，逆に顕著な発育促進作用のみが認められた．即ち静置培養では48時間目の optical density は Cirate

濃度に比例して大となつた．又振盪培養では対数増殖期曲線の傾斜が急でこの分裂速度の増大が認められ，一つ有機酸無添加の場合の最大発育期たる18時間目に optical density は無添加の場合に比して3倍強に達つていた（第6図）．

Fig 6 第6図　Citrate の Salmonella typhi の発育に及ぼす影響



註：第1図註に同じ

考　察

偏性嫌気性菌は振盪培養により菌の発育が良好となることは既に知られて居り，又振盪培養により菌の発育が旺盛になる現象に関して三橋[1]等は主として培地内の酸素分圧の増加を挙げているが振盪により培地表面積が増大することも無視出来ない．Heukelekian and Heller[2] は Escherichia coli を用いて培地表面積の拡大が菌の発育に促進的影響を与えることを認め，培地と固体或は培地と空気との界面に陽性荷電の栄養物質が集積することが以上の現象を生ずる原因であると考えている．増井等[3]は此の現象を応用し培養の困難であつた Hemophilus pertussis の培養に際し，培地に硝子球を投入し培地面積を増大させて好結果を得ている．従つて振盪によつて培地と空気の界面の面積が増加することも振盪培養が静置培養に比較して菌の増殖に好影響を与えているのであろう．筆者の実験から Escherichia coli に対しては Succinate 及び Fumarate が，Salmonella typhi に対しては Succinate 及び Citrate が振盪培養の場合も静置培養の場合も発育促進的に働いていることは明らかである．

上述の原因としては先づ Escherichia coli は Succinate, Fumarate の，Salmonella typhi は Succinate, Citrate の合成能が旺盛な発育を営むに充分なだけ発揮されないのではあるまいかと考えられる．

T.C.A.-cycle 関与有機酸は Glucose と異り直接力源として利用される可能性がある．しかも両菌株共に T.C.A.-cycle 関与酵素系の存在が証明されているから[9-16] Succinate, Fumarate 及び Citrate が力源として直接利用されるという可能性は否定出来ない．然し乍ら之等有機酸が力源として利用されるには主として菌体内に於ける酵素量と菌体内への有機酸の透過性が関与すると考えられる．従つて両菌株間に同一濃度の有機酸でも発育促進効果に差があるのは当然と云えよう．又 Citrate は Escherichia coli に対しては高濃度ではむしろ発育阻害的に働くのに，Salmonella typhi に対しては顕著な発育促進作用が認められたことは極めて興味深いことである．

又静置培養の場合は一般に有機酸の添加無添加に拘らず最大発育に至る迄の時間が長いが，振盪培養では一般に短く特に有機酸を添加した場合に著明に認められた．此の事は菌体の適応酵素の産生が静置培養では遅れていることを意味し且つ力源の獲得に要する時間の差異も存すると考えられよう．

結　論

Escherichia coli 及び Salmonella typhi を用ひ振盪培養と T.C.A.-cycle 関与有機酸中 Sodium succinate, Sodium fumarate 及び

Sodium citrate の発育に及ぼす影響を検し次の結果を得た。

1. 振盪培養を行うと静置培養の場合に比し optical density にして約2倍の発育が認められた。

2. Escherichia coli は Sodium succinate 或は Sodium fumarate を添加することに依り著明な発育促進作用が認められた。

3. Salmonella typhi は Sodium succinate 或は Sodium citrate を添加することに依り著明な発育促進作用が認められた。

本稿を終えるに当り終始御懇篤な御指導御校閲を賜はつた桑島教授・増井助教授に深甚の謝意を捧げる。又実験に当つて常に熱心に助力して下さつた久保光子君に厚く謝意を表する。

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諸種細菌の振盪培養と T. C. A.-cycle 關與有機酸の発育に及ぼす影響

I Staphylococcus Aureus 及び Mycobactrium Avium

Bacterial Growth by Shake Culture Method and a Growth Stimulating Effect of Some Organic Acids Related to T.C.A.-cycle

I Staphylococcus Aureus and Mycobacterium Avium

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（昭和29年3月11日受付）

緒　言

第1報[1]に於ては E.coli 及び Salmonella ryphi を用いて振盪及び T. C. A.-cycle 関与有機酸の発育に及ぼす影響を検したが，本報に於ては Staphylococcus aureus 及び Mycobacterium avium に対する振盪及び有機酸の発育に及ぼす影響を検したので報告する。

I 実験方法

（1）使用菌株及び供試培地：当教室保存の Staphylococcus aureus 209p 及び Mycobacterium avium 竹尾株を用いた。

Staphylococcus aureus には基礎培地としてCasamino acids technical Difco 2.0g, K_2HPO_4 2.0g, Glucose 2.0g, Cysteine hydrochloride 0.1g, Nicotinic acid 4.0mg, Thiamine 5.0mg, NaCl 2.0g, $MgSO_4$ 0.1g を 1L. 蒸溜水に溶解して pH 7.0 とし，高圧滅菌して使用した。Mycobacterium avium には基礎培地として Citric acid を除いた Sauton 培地を用いた。実験に際し有機酸を種々の濃度になるように，それ等の Seitz 濾液を基礎培地に加えた。

（2）増殖及び成績の判定：Staphylococcus aureus の場合は培養及び成績の判定は第1報[1]に準じて行つた。Mycobacterium avium の場合は次の如くにして行つた。即ち Sauton 培地約1週間培養菌体で手振法により 1.25mg/ml の菌液を作り，予め滅菌小試験管に 2ml 充分注した培地に1滴宛（約).05mg）に滴下し，37°C で1週間静置及び振盪培養を行つた。振盪は振幅 3.5cm 振盪回数 2.0cycle/min で行つた。培地表面に菌膜の認められる第2日目より隔れ隔日に菌体を遠心分離後3回生理食塩水で洗滌し，micro-Kjeldahl 法により菌体窒素量を測定した。基礎培地として Citric acid を除いた Sauton 培地を用いたが，実験に際しては Succinic acid, Fumaric acid, Malic acid, Citric acid, α Ketoglutaric acid, Lactic acid 及び Pyruvic acid の Na 塩溶液の Seitz 濾液を各種濃度になるように基礎培地に加えた。

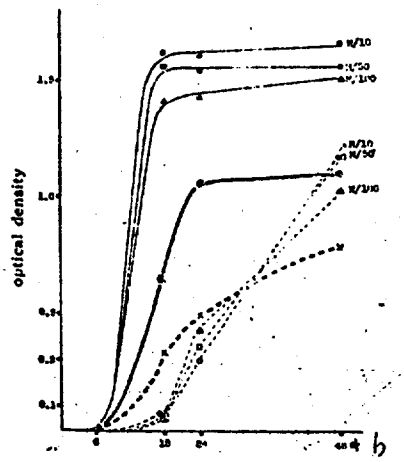

Figure 1

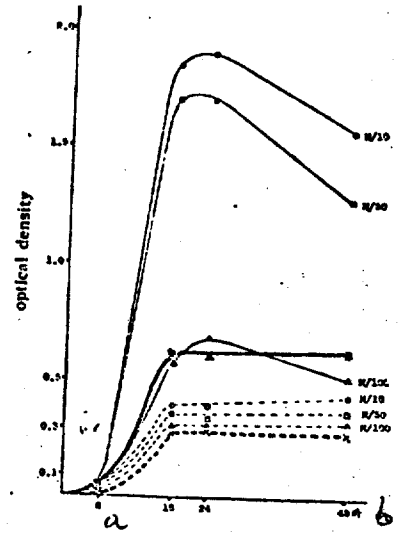

Figure 2

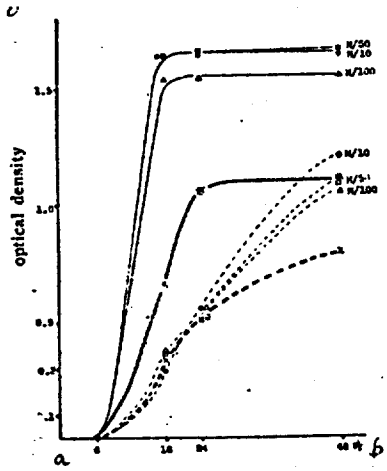

Figure 3

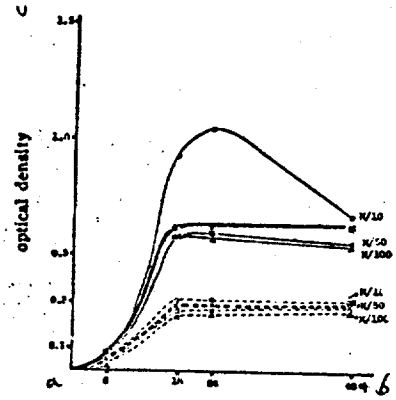

Figure 4

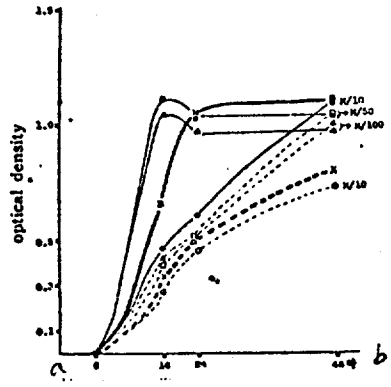

Figure 5

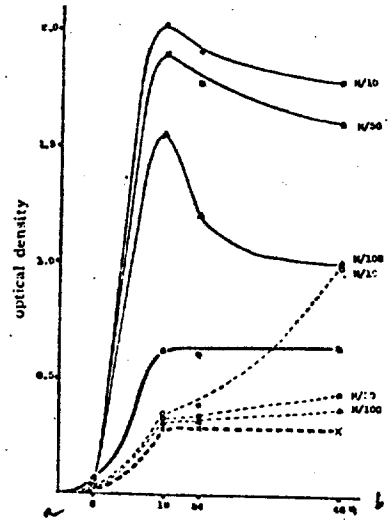

Figure 6

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Secretary, Mr. H. Chapman, is the Chairman. The Fair Prices Defence Committee have pursued with energy and by all available means the objective which is the sole reason for its existence and has become recognised as one of the foremost champions of the case for price-maintenance. Each of the constituent organisations is likely to call on its branches, local associations and individual members to co-operate in the campaign, with special reference to the circumstances and factors affecting their particular trade.

BIOLOGICAL STANDARDISATION

MUCH water has flowed beneath the bridges since the Health Organisation of the ill-fated League of Nations established the first International Biological Standards. As every student of the subject is taught, this action was the trigger-mechanism for a series of events, not the least of which was the passing of the Therapeutic Substances Act, 1925, with its schedules of medicinal substances which require to be standardised by biological methods. A review (and pre-view) of biological standardisation has recently appeared in our columns. This has served to refresh the minds of pharmacists, especially those concerned with "screening" new compounds, and assaying old ones, of the part played by the Department of Biological Standards of the Medical Research Council, at Mill Hill, N.W.7. It may, however, be less well known to our readers that this Authority acts under an even greater one, namely, the W.H.O. of the United Nations. In fact, Mill Hill provides the Chairman (Dr. A. A. Miles) and a biological statistician—may we say biometrician—(Dr. W. L. M. Perry) towards an Expert Committee on Biological Standardisation, of which the Secretary is Dr. W. Aeg. Timmerman.

In its report on the Fourth Session held at Geneva in November, 1950, summarised on p. 489 of this issue, the Committee has issued notes on twenty-four therapeutic substances, oxophenarsine, dimercaprol and [illegible] include nine bacterial agents, with a topical [illegible] of B.C.G. vaccine centres, a review of existing standards on the hormones, organoarsenicals, vitamin B_{12}, the A and D standards, and authority to proceed to establish the pro-vitamin A standard. A new international penicillin standard is envisaged as well as those for aureomycin, terramycin and chloramphenicol. Other antibiotics have (says the report) not yet reached the stage to justify the establishment of an International Standard. Nevertheless, in order to encourage and regularise research, a collection of "Author's preparations" is mooted, and an invitation will be issued to workers to contribute their "laboratory standards" to this collection. Furthermore—and here we gladly lend our aid—"When any other unit of potency (i.e., other than International) is cited, it should be fully described in the context or proper references made to a published description." Three important paragraphs (18, 19, and 20) call for special mention. They deal with tuberculosis and tuberculin. In the first, the Expert Committee lays down minimum requirements of equipment and personnel for "laboratories engaged in the demonstration of tubercle bacilli," and promises a list of places where proper training in the diagnosis of tuberculosis can be obtained. The twentieth "note" comes near to the pharmacist's heart, in promising to advise the Expert Committee on the Unification of Pharmacopœias, instancing for the present, pyrogenic substances, oxophenarsine, dimercaprol and D-tubocurarine.

In the final paragraph the Committee pleads that "in addition to publicising them in the various periodicals of W.H.O., information about them should be brought to the notice of editors of general medical journals and of journals devoted to special fields of work in which the use of a particular standard was relevant." Accepting this rôle, THE PHARMACEUTICAL JOURNAL hopes to play its part in this worth-while campaign.

VOLUMETRIC DETERMINATION OF SODIUM CITRATE

By R. J. JERAM and P. COOPER

THIS method has been developed for use as a routine check on solutions of sodium citrate for injection. The pharmacopœial method would involve evaporation of about 50 mils of solution to dryness, followed by incineration, acidification, boiling and back-titration which is a lengthy procedure. The principle here is precipitation of the silver salt in neutral solution by a known excess of silver nitrate and reduction of its solubility by the addition of acetone. The precipitate is filtered off and the excess silver nitrate in the filtrate back-titrated with a standard thiocyanate.

The following points were ascertained as a preliminary:

1. Both a 4 per cent. solution of sodium citrate and a $N/10$ solution of silver nitrate give clear solutions with an equal volume of acetone. Therefore, the addition of acetone up to 50 per cent. to the test solution does not produce unwanted precipitation of the reagents.

2. The solubility of silver citrate is at a minimum over a *p*H range of 7 to 8. A slight rise of acidity greatly increases the solubility, and precipitation is not uniform unless the solution of sodium citrate is neutral or slightly alkaline. In the presence of acid substances, other than traces of citric acid, the *p*H of the solution should be adjusted before adding the silver reagent until a bluish tinge is produced with bromothymol blue indicator.

3. A sintered-glass filter of No. 3 porosity is adequate for removing the precipitate, provided the mixture of citrate and silver reagent is boiled before filtration, to coagulate the silver citrate.

4. Washing of the precipitate with distilled water causes loss of silver, and this is minimised by using 50 per cent. acetone in distilled water and a small volume of washings.

5. Titration of either the filtrate or the precipitate, after treatment with excess nitric acid, may be used as a basis for calculation. It is more convenient to use the filtrate, however, employing ferric alum as internal indicator. The error should be not more than 0·5 per cent. by the following technique:

TECHNIQUE

(*a*) Applicable to a nominally 3·8 per cent. neutral solution of sodium citrate.

1. Take 50 mils and dilute to 250 mils with water.
2. Take 10 mils of the dilution and add 20 mils $N/10$ silver nitrate. Bring to the boil and allow to stand about one minute.
3. Add 30 mils acetone cautiously, and shake. Allow to stand one minute and filter through a No. 3 sintered-glass filter or glass-wool plug.
4. Wash the precipitate with 50 mils of 50 per cent. acetone, in four portions.
5. To the filtrate and washings add 1 mil nitric acid and a little ferric alum.
6. Titrate with $N/10$ ammonium thiocyanate to the orange end point.
7. Each mil $N/10$ silver nitrate reacting with the citrate is equivalent to 0·009803 gm. of $C_6H_5O_7Na_3, 2H_2O$.

(*b*) Applicable to the dry salt.

About 2 gm. accurately weighed is dissolved in water and made up to 250 mils. Ten mils of this solution is used as (2) above.

Submitted from the Pharmaceutical Department, Saint Mary's Hospital, Portsmouth, by kind permission of the Medical Superintendent.

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Papers of the Academy of Sciences of the Ukrainian SSR, No. 9
Series B. Geology, Geophysics, Chemistry and Biology

Biochemistry of people and animals

S. B. Komisarenko and Academician of the Ukr. Academy of Sciences M. F. Hulyj.

The influence of citric acid on the incorporation of glycine-1-C^{14} into rabbit liver and muscle proteins in vitro.

Based on the discovery of the transamination process /1/, as well as our own research (which proved the formation of glutamic acid from ketoglutaric acid by means of the glutaminodehydrogenase enzyme in the presence of ammonium salts), Euler, together with his collaborators in 1937, expressed the idea that the tricarboxylic cycle definitely plays an important role in the formation of some amino acids /2/. Later it had been established /3/ that the presence of ammonium salts facilitates the formation of glutamic and aspartic acids at the expense of citrate in the animals' livers.

Almost all of the amino acids (with an exception of lysine, threonine and arginine) may be formed from the appropriate ketoacids by means of transamination in which the most important role is played by the components of the tricarboxylic cycle, i. e.: oxaloacetic and especially the α-ketoglutaric acids. The intermediates of the citrate cycle also take part in the biosynthesis of other important biological compounds, for example, the aspartic acid is used for the biosynthesis of purines and pyrimidines /4/; α-ketoglutaric acid (or succinate) through the glycinosuccinate cycle of Shemin takes part in the formation of porphyrines and purines /5/.

Departing from the position that the Krebs cycle directly or indi-

rectly, is connected with the formation of the amino acids of the purine and pyrimidine bases (and thus also of nucleic acids) and taking into account the fact that the basic fundtion of the cycle is the production of ATP (and through it of other nucleosidetriphosphates) it is to be expected that the cycle definitely influences the biosynthesis of the protein in the cell.

In our experiments we tried to find out the influence of various doses of citrate upon the incorporation of the glycine-1-C^{14} into the proteins of liver slices and into the diaphragm muscle of rabbits. We have also determined the actual radioactivity of lipoids of these slices.

Our experiments were conducted on the liver slices and the diaphragm muscle in a Warburg apparatus for 75 minutes at 37° C, open to (the oxygen of) the atmosphere. Into the main space of the ware we brought 2.2 ml of the agent prepared with Krebs-Ringer phosphate buffer, pH 7.2, and in the central tube there was 0.2 ml 10% KOH. Into the side tube, in addition to 0.1 ml of solution of glycine-1-C^{14} (0.154 mg of dry glycine with 2 mc activity) we added 0.5 ml of sodium citrate solution which contained 4 μM solution into the experimental sample, and 0.5 ml of the agent into the control sample. In the experiment with liver slices, in addition to the citrate dose of 4 μM, we also investigated the influence of 8 μM and 20 μM doses. The overall quantity of solution in the ware was 3.0 ml and the incubated mixture - 2.8 ml. Within 15 minutes when the temperature in the wares was balanced, the contents of the side branches was poured into the central space, and within an additional 75 minutes the incubation was terminated by the introduction of 10% trichloroacetic

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acid. Respiration of the slices was observed for one hour. Lipoids were extracted with a chloroform-methanol mixture and the proteins were freed from nucleic acids by heating below 90° C for 25 minutes with 10% trichloroacetic. We reprecipitated with 88% formic acid, rinsed with trichloroacetic acid, water, alcohol, and finally, ether and dried to constant weight /6/. We placed proteins and lipoids on standard targets, measured their radioactivity with the T-25 BFL computer and the PP-8 radiometer and calculated the actual radioactivity of the samples in impulses/minute/mg of solution taking into consideration the background and self-absorption factors.

Our data have been calculated statiscally by the differential method /7/.

In spite of the fact that the citrate penetrates the cells to a very small extent, we have arrived at a statistically reliable stimulation by the citric acid of the incorporation of glycine-1-C^{14} into proteins and the carbon C^{14} into lipoids of the liver slices and diaphragm muscle. Thus citrate doses of 4 μM and 8 μM increased the incorporation of the glycine-1-C^{14} into the proteins of liver slices by 37% and 29% respectively. (See Table 1)

Table 1

Incorporation of glycine-1-C^{14} into proteins of liver slices in impulses/minute/mg.

	Control	Citrate 4 μM	Control	Citrate 8 μM	Control	Citrate 20 μM
M	79	109	100	129	118	113
n	7		7		7	
%	100	137	100	129	100	95
	p<0.01		p<0.02		p<0.5	

A 20 μM dose not only failed to stimulate the inclusion of radioactive tags but also sometimes decreased it.

There are controversial research data concerning the influence of citrate upon the incorporation of radioactive tags into lipoids of the slices, obviously because of the poor absorption of the citric acid into the cell /8-1/. However these studies used acetate-C^{14} as the tagging device. In our experiments, on the other hand, glycine had to convert previously into a substrate in order to participate in the lipogenesis. Yet the citrate, although insignificantly, stimulated the inclusion of the radioactive tag into lipoids in the same concentration as in the protein.

The 20 μM dose appeared to be ineffective. (See Table 2)

Table 2

The actual radioactivity of lipoids of the liver slices in impulses/minute/mg.

	Control	Citrate 4 μM	Control	Citrate 8 μM	Control	Citrate 20 μM
M	507	577	452	505	560	550
n	7		6		7	
%	100	114	100	112	100	98
	p<0.01		p<0.01		p<0.5	

On the slices of diaphragm muscle we compared only the activity of 4 μM of citrate (see Table 3) with the control group and we observed the increase of the actual radioactivity of proteins and lipoids of the tested samples which was 45% and 15% respectively compared with the control material.

It is to be stated that during the recorded time the respiration level of the slices remained stable and did not vary either in the test or in the control wares.

The inclusion of glycine-1-C^{14} into proteins of the liver slices and of the diaphragm muscle which we have acquired in our experiments through the stimulation by citric acid, of course, can be explained by the activity of the doses employed (4 and 8 μM) upon the tricarboxylic acid cycle and thereby the intensification of the regeneration which is necessary for the biosynthesis of the ATP protein. It is also possible that the positive influence of the oxaloacetic and α-ketoglutaric acids which appear in the cycle as predecessors of the aspartic and glutamic acids. These latter take part in the formation of other amino acids through transamination.

The decrease of the radioactive tag concentration with an increase of the citrate concentration (20 μM) occurs, for certain, because the citrate performs here a substratal function and causes the dilution of the tagged material.

Our research on the influence of various concentrations of sodium citrate upon the inclusion of glycine-1-C^{14} into the proteins and upon the actual radioactivity of lipoids of the liver slices and diaphragm muscle slices of rabbits have shown that sodium citrate in the doses of 4 μM and 8 μM stimulates the inclusion of the radioactive tag into proteins and lipoids of the liver slices. The 20 μM dose does not substantially change the inclusion of the isotope. 4 μM of citrate increases the actual radioactivity of proteins and lipoids of the diaphragm muscle slices.

The citrate doses employed did not change the tissue respiration level.

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Table 3

The actual radioactivity of proteins and lipoids of the diaphragm muscle.

	Proteins		Lipoids	
	Control	Citrate 4 μM	Control	Citrate 8 μM
M	22	32	36	41
n	5		6	
%	100	145	100	115
	p<0.01		p<0.001	

1969 ДОПОВІДІ АКАДЕМІЇ НАУК УКРАЇНСЬКОЇ РСР № 9
СЕРІЯ Б. ГЕОЛОГІЯ, ГЕОФІЗИКА, ХІМІЯ ТА БІОЛОГІЯ

Біохімія людини та тварин

УДК 577.1:612.015.32

С. В. КОМІСАРЕНКО і акад. АН УРСР М. Ф. ГУЛИЯ

ПРО ВПЛИВ ЛИМОННОЇ КИСЛОТИ НА ВКЛЮЧЕННЯ ГЛІЦИНУ-1-C^{14} У БІЛКИ ПЕЧІНКИ ТА М'ЯЗА КРОЛІВ IN VITRO

Грунтуючись на відкритті процесу трансамінування [1], а також на власних дослідженнях, які показали утворення глютамінової кислоти з α-кетоглютарової за допомогою ферменту глютамінодегідрогенази у присутності солей амонію, Ейлер з співробітниками в 1937 р. висловив думку про те, що трикарбоновий цикл, очевидно, відіграє важливу роль при утворенні деяких амінокислот [2]. Пізніше з'ясовано [3], що додавання солей амонію сприяє утворенню глютамінової і аспарагінової кислот за рахунок цитрату в печінці тварин.

Майже всі амінокислоти (за винятком лізину, треоніну і аргініну) можуть утворюватися з відповідних кетокислот трансамінуванням, де найважливішу роль відіграють члени трикарбонового циклу — щавлевооцтова і, особливо, α-кетоглютарова кислоти. Інтермедіанти цитратного циклу беруть також участь в біосинтезі інших важливих біологічних сполук. Так, аспарагінова кислота використовується для біосинтезу пуринів і піримідинів [4]; α-кетоглютарова кислота (чи янтарна) через гліцинносукцинатний цикл Шеміна бере участь в утворенні порфіринів та пуринів [5].

Виходячи з того, що цикл Кребса прямо чи опосередковано пов'язаний з утворенням амінокислот, пуринових і піримідинових основ (а, отже, і нуклеїнових кислот) і враховуючи те, що основною функцією циклу є генерування АТФ (а через неї й інших нуклеозидтрифосфатів), можна чекати, що цикл істотно впливає на біосинтез білка в клітині.

В наших експериментах вивчався вплив різноманітних доз цитрату на включення гліцину-1-C^{14} в загальні білки зрізів печінки і діафрагмального м'яза кролів. Визначали також і питому радіоактивність ліпідів цих зрізів.

Експерименти провадились на зрізах печінки і діафрагмального м'яза кролів, які добу голодували. 400 *мг* зрізів інкубували в посудинах апарата Варбурга 75 хв. при 37° С в атмосфері кисню. В основний простір посудини вносили 2,2 *мл* середовища, приготованого на Кребс—Рінгер фосфатному буфері pH 7,2; у центральній склянці було 0,2 *мл* 10% КОН. У боковий відросток крім 0,1 *мл* розчину гліцину-1-C^{14} (0,154 *мг* сухого гліцину з активністю 2 *мккюрі*) додавали: в дослідні проби по 0,5 *мл* розчину цитрату Na, який містив 4 *мкМ* речовини, а в контрольні — по 0,5 *мл* середовища. В експериментах зі зрізами печінки, крім дози цитрату в 4 *мкМ*, досліджували вплив 8 *мкМ* і 20 *мкМ*. Загальний об'єм рідини в посудині становив 3,0 *мл*, а інкубаційної суміші — 2,8 *мл*. Через 15 хв., коли температура у посудинах була зрівноважена, вміст з бокових відростків вилився в основний простір, а ще через 75 хв. інкубація припинялась додаванням 10% трихлороцтової кислоти. Дихання зрізів фіксувалося протягом години. Ліпіди екстрагували хлороформметаноловою сумішшю, а білки

вивільняли від нуклеїнових кислот прогріванням протягом 25 хв. з 10% трихлороцтовою кислотою при 90° С; переосаджували 88% мурашиною кислотою, промивали трихлороцтовою кислотою, водою, спиртом, ефіром і висушували до постійної ваги [6]. Білки і ліпіди наносили на стандартні мішені, вимірювали їх радіоактивність торцевим лічильником Т-25 БФЛ за допомогою радіометра ПП-8, розраховували питому радіоактивність зразків в *імпульсах/хв/мг* речовини з урахуванням фону і самопоглинання.

Дані опрацьовували статистично різницевим методом [7].

Незважаючи на те, що цитрат погано проникає в клітини, ми одержали статистично вірогідне стимулювання лимонною кислотою включення гліцину-1-C^{14} в білки і вуглецю C^{14} у ліпіди зрізів печінки і діафрагмального м'яза. Так, дози цитрату 4 *мкМ* і 8 *мкМ* на пробу збільшували включення гліцину-1-C^{14} у білки зрізів печінки відповідно на 37% і 29% (табл. 1).

Таблиця 1

Включення гліцину-1-C^{14} у білки зрізів печінки в *імп/хв/мг*

	Контроль	+4 *мкМ* цитрату	Контроль	+8 *мкМ* цитрату	Контроль	+20 *мкМ* цитрату
M	79	109	100	129	118	113
n	7		7		7	
%	100	137	100	129	100	95
	$p<0{,}01$		$p<0{,}02$		$p<0{,}5$	

Доза 20 *мкМ* не лише не стимулювала включення мітки, але й інколи зменшувала її.

Існують суперечливі літературні дані про вплив цитрату на включення мітки у ліпіди зрізів, очевидно, через погане проникнення лимонної кислоти в клітину [8—10]. Проте, в цих роботах міткою служив ацетат-C^{14}. У наших же експериментах гліцин повинен був попередньо перетворитися на субстрат для участі в ліпогенезі. І все ж таки цитрат, хоч і незначно, але стимулював включення мітки у ліпіди тими ж концентраціями, що і в білок.

Доза 20 *мкМ* тут також виявилась неефективною (табл. 2).

Таблиця 2

Питома радіоактивність ліпідів зрізів печінки в *імп/хв/мг*

	Контроль	+4 *мкМ* цитрату	Контроль	+8 *мкМ* цитрату	Контроль	+20 *мкМ* цитрату
M	507	577	452	505	560	550
n	7		6		7	
%	100	114	100	112	100	98
	$p<0{,}01$		$p<0{,}01$		$p>0{,}5$	

На зрізах діафрагмального м'яза порівнювалась з контролем дія тільки 4 *мкМ* цитрату (табл. 3), і було відмічено збільшення питомої радіоактивності білків і ліпідів дослідних проб відповідно на 45 і 15% порівняно з контрольними.

Слід зазначити, що протягом фіксованого часу рівень дихання зрізів залишався стабільним і не відрізнявся в дослідних і контрольних посудинах.

Одержане у наших експериментах стимулювання лимонною кислотою включення гліцину-1-C^{14} у білки зрізів печінки і діафрагмального м'яза можна, очевидно, пояснити активуючою дією вивчених доз (4 і 8 *мкМ*) на трикарбоновий цикл і підсиленням таким чином регенерації, необхідної для біосинтезу білка АТФ. Можливий також позитивний вплив виникаючих у циклі щавлевооцтової і α-кетоглютарової кислот, як попередників аспарагінової і глютамінової кислот. Останні беруть участь в утворенні інших амінокислот переамінуванням.

Таблиця 3

Питома радіоактивність білків та ліпідів діафрагмального м'яза

	Білки		Ліпіди	
	Контроль	+4 *мкМ* цитрату	Контроль	+4 *мкМ* цитрату
M	22	32	36	41
n	5		6	
%	100	145	100	115
	$p<0{,}01$		$p<0{,}001$	

Зниження мітки, із збільшенням концентрації цитрату (20 *мкМ*) відбувається, напевне, через те, що цитрат тут виконує субстратну функцію і викликає розведення мітки.

Дослідження впливу різних концентрацій цитрату Na на включення гліцину-1-C^{14} у білки і на питому радіоактивність ліпідів зрізів печінки і діафрагмального м'яза кроликів показали, що цитрат Na дозою 4 і 8 *мкМ* на пробу стимулює включення мітки в білки і ліпіди зрізів печінки. Доза 20 *мкМ* істотно не змінює включення ізотопу. 4 *мкМ* цитрату збільшують також питому радіоактивність білків і ліпідів зрізів діафрагмального м'яза.

Досліджувані дози цитрату не змінювали рівня дихання зрізів тканин.

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ON THE CITRIC ACID INFLUENCE ON THE GLYCINE—1—C^{14} INCORPORATION INTO RABBIT LIVER AND MUSCLE PROTEINS IN VITRO

Summary

The effect of various citrate concentrations on the glycine-1-C^{14} incorporation into protein and on the lipid specific radioactivity of rabbit liver and muscle slices was studied.

It was found out that sodium citrate in doses of 4 μM and 8 μM stimulates the tracer incorporation into liver slices proteins and lipids. 20 μM does not influence this incorporation. 4 μM of citrate also increases the specific radioactivity of muscle proteins and lipids.

The investigated citrate concentrations do not influence the oxygen consumption.

CITRATES NO. 214

Kuo, C. Y. et al. 1965. The effect of x-irradiation on Escherichia Coli B. II. Inhibiting the role of deoxyribonucleace (DNase) in the degredation of DNA in Escherichia Coli after x-irradiation. Yuan Tzu Neng. 3:245-250

Table I. Determination of Known Amounts of Arsenic Added to Soil[a]

Arsenic Added, γ	Arsenic Found, γ	Average, γ	Average, P.P.M.
1.0	1.0, 1.0, 1.0, 1.0, 1.5	1.1	11
2.5	3, 3, 3, 2, 3	2.8	28
5.0	5, 4, 5, 4, 5	4.6	46
12.0	12, 12, 12, 13, 12	12.1	121
20.0	19, 20, 18, 17, 16	18	180
40.0	40, 40, 30, 40, 25	35	350

[a] Analyses by K. T. Williams and R. R. Whetstone, U. S. Department of Agriculture. The 0.1-gram samples used contained 0.03γ of arsenic; this amount would have a negligible effect on the determination of added arsenic.

add 0.5 ml. of 10% stannous chloride solution, and dilute to 10 ml. with water. Add a 2- to 4-gram piece of mossy zinc, and quickly insert the rubber stopper holding the glass pipe of the Gutzeit apparatus. Let stand 1 hour. Remove the mercuric chloride paper and compare with standard artificial spots. Determine the arsenic content of samples containing more than 100 p.p.m. by transferring a smaller aliquot to the test tube, adding 2 ml. of concentrated hydrochloric acid, and continuing as directed in the procedure.

RESULTS

In the ranges of arsenic concentrations of importance for prospecting, the precision and dependability of the field method are shown in Table I. Known quantities of arsenic were added to samples of soils of negligible arsenic content, and arsenic was determined by the field method. These experiments show that the simple field method gives reproducible values close to the true arsenic content and should be useful in outlining anomalies in arsenic content.

A series of soil samples was collected at increasing depths from the Blackbird area, Idaho, and analyzed for cobalt and arsenic. Both cobalt content and arsenic content increased with increasing depth. As shown in Table II, the increase in cobalt content from the surface to a depth of 12 feet at location A is 700 p.p.m., whereas the increase in arsenic is 7600 p.p.m. These data indicate that determination of arsenic in soils may be ir[illegible] finding ore in certain localities.

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Table II. Increase in Cobalt and Arsenic with Depth in Soils Overlying Cobalt Ore

Location	Depth of Sample, Feet	Cobalt, P.P.M.	Arsenic, P.P.M.
A	0	100	400
	2	400	2000
	4	300	4000
	12	800	8000
B	0	70	300
	2	200	700
C	0	55	100
	2	100	600

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Rapid Accurate Determination of Carbohydrates and Other Substances with the Dichromate Heat-of-Dilution Method

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A simple dichromate oxidation technique was recently developed for the determination of moisture in fruits and vegetables (*5, 8*). The oxidation values for such foods were found to be very reproducible (standard deviations, 0.1 to 0.3%), and the oxidation values for standard samples (National Bureau of Standards) of glucose and sucrose were close to theory. The technique dispenses with the usual external heating by utilizing the heat of dilution of concentrated sulfuric acid, and is thus very rapid, requiring a total time of about 5 minutes per weighed specimen. Thus, the technique should find application in the rapid and accurate determination of certain types of pure organic substances in the presence of water and other nonoxidizables, or in the analysis of binary mixtures of pure substances, or as a simple aid in the determination of chemical composition.

This report deals with an investigation of the scope and limitations of the technique, as applied to starch, typical sugars, and various other types of organic substances. Application to various types of celluloses, involving special problems, has been described (*4*).

METHOD

All operations were performed in watch-glass covered beakers by using the following procedure.

The test specimen, calculated to reduce 80%, approximate upper limit, of the dichromate—for example, 0.15 gram of benzoic acid or 2.5 grams of sodium oxalate—was weighed out and 25.00 ml. of 1.835*N* potassium dichromate was added. (This solution contained 90.00 grams per liter of reagent-grade potassium dichromate crystals, dried overnight at 100° to 140° C. The solution was filtered before use, and no other precautions were found necessary in order to secure within 0.05% of nominal concentration when the solution was standardized against National Bureau of Standards oxidimetric standard potassium dichromate.) Then 10 ml. of concentrated sulfuric acid, reagent grade, d. 1.84, was slowly added (10 to 15 seconds) to the well-stirred solution or mixture. Efficient stirring without splashing, easily accomplished by means of a magnetic stirrer, was required to avoid reduction of the sulfuric acid. After foaming had moderated, which usually required 15 seconds, 30 ml. more of the acid was rapidly added. After 10 minutes, 150 ml. of water was added and the excess dichromate was titrated whenever convenient. Actually, a 3-minute standing time before titration was found to give maximum yield for typical carbohydrates, aliphatics, and most aromatics, with the exceptions of anthranilic acid and nitrophenol, which gave 0.5% lower yield at the 3-minute point. However, 10 minutes permitted the most efficient utilization of time in the analysis of series of replicates. Blank determinations gave no reduction in titer.

The excess dichromate was determined by electrometric titration of the entire solution without further dilution, using 0.5*M* ferrous ammonium sulfate (0.2*M* in sulfuric acid). This solution is not stable and should be standardized against the dichromate

Table I. Results on Starch, Sugars, Aliphatics, and Aromatics

Substance	Yield, % of Theoretical	Standard Deviations	No. of Replicates
Starch	99.35	0.21	10
Glucose	99.40	0.12	12
Fructose	99.40	0.17	10
Sucrose	99.25	0.09	10
l-Arabinose	99.60	0.12	6
l-Arabitol	99.35	0.18	10
α-Methyl mannoside	99.25	0.10	8
Galacturonic acid.H_2O	99.60	0.15	10
Methyl galacturonide.$2H_2O$	99.40	0.14	6
Methyl galacturonide Me ester.H_2O	99.45	0.17	10
Trimethyl methyl mannoside	99.65	0.06	5
Formic acid	99.95	0.15	8
Oxalic acid (Na salt)	100.00	0.04	9
Glycolic acid	99.85	0.10	7
Methanol	99.50	0.34	5
Glycerol	100.00	0.13	6
Malonic acid	99.85	0.08	6
Tartaric acid (Na salt.$2H_2O$)	99.60	0.07	10
Maleic acid	99.85	0.07	9
Citric acid (Na salt.$2H_2O$)	99.35	0.13	10
Inositol	99.30	0.15	10
Benzoic acid	99.30	0.34	10
Phthalic acid (KH salt)	99.25	0.16	6
Anisic acid	98.60	0.40	6
Anthranilic acid	99.05	0.18	6
p-Nitrophenol	98.65	0.35	6

solution before and after a series of titrations. A simple procedure was to add 5.00 ml. of the dichromate solution to a solution which had just been titrated, and then to retitrate to the same end point. Commercial titrating equipment may be used; the writers prefer a simple arrangement consisting of an inexpensive microammeter (1 μa. per division), a radio potentiometer (500 to 5000 ohms), a 6-volt dry battery, and a tungsten, Nichrome, or calomel electrode versus a positive platinum electrode. Additional details and a discussion of other dichromate methods have been reported (*5*).

MATERIALS

The sucrose, sodium oxalate, and benzoic and anisic acids were standard samples from the National Bureau of Standards and were used without corrections for moisture and ash. The fructose was a purified commercial product.

The starch was a highly purified material prepared from potatoes by A. L. Potter of this laboratory, and was found to have 0.004% amino nitrogen. Ash was 0.25%. Moisture was determined with a vacuum oven at 3 mm. of mercury and 100° C. for 5 hours.

The rest of the sugars were prepared and purified by R. M. McCready of this laboratory. The other substances were the best obtainable commercially and were used without further purification. Ash and moisture values were in general very low. Ash was determined, after precharring, at 550° C., usually for 16 hours. Moisture was determined for the other substances with the Karl Fischer reagent, with the following exceptions: Fructose, nonhygroscopic in any case, was stored for 6 days in vacuo over anhydrous magnesium perchlorate and used without moisture correction. The moisture in arabinose and arabitol was measured with a vacuum oven at 3 mm. of mercury and 60° C. for 16 hours, that in sodium citrate with a circulating-air oven at 105° C. for 1 hour, and that in formic acid, by titration. Carbon and hydrogen combustion analyses were made on trimethyl methyl mannoside. A small amount of sodium hydroxide solution was added to the benzoic, anisic, and anthranilic acids before addition of dichromate, as they are not sufficiently soluble in, or easily wetted by, the reagent.

RESULTS AND DISCUSSION

The results obtained by applying the method to various types of substances are shown in Tables I and II. The yield values were based upon the assumption that dichromate is reduced to the chromic state (Cr^{+++}), that all carbon is oxidized to carbon dioxide, and that no other valence changes occurred (with the one exception of nitrophenol, whose nitrogen is oxidized from +3 to +5).

The following is a typical calculation of yield: 1.0000 gram of *l*-arabinose, containing 0.10% ash and 0.15% moisture, reduced 72.11 ml. of 1.835*N* dichromate. This becomes 72.29 ml. on the dry, ashless basis, and gives 72.29 × 0.001 × 1.835 × 150.13 (formula weight of arabinose) = 19.915 equivalents per mole. Since complete oxidation to carbon dioxide corresponds to 20 equivalents per mole, the yield is 19.915/20 = 0.9960, which is expressed in per cent in Table I. The yields are mean values of the indicated numbers of replicates and the standard deviations refer to deviations of individual values from the means. The yields are expressed to the nearest 0.05%, as that was the approximate combined titrational uncertainty in a mean of six replicates, exclusive of uncertainties in the moisture and ash determinations. These yields may be compared with others for a variety of substances using external heating methods (*1–3, 6, 7*).

Table II. Examples of Incomplete Oxidation

Substance	Yield, % of Theoretical	Standard Deviations	No. of Replicates
With CH_3—or CH_2 groups			
Ethyl alcohol	35	1.2	6
Acetic acid (Na salt)	3	9	6
Propionic acid	62	1.2	6
Succinic acid (Na salt.$6H_2O$)	60	5	9
Malic acid	94	0.8	10
Lactic acid (Ca salt)	38	0.9	6
Lauric acid	72	2	10
Stearic acid (Na salt)	46	8	5
l-Rhamnose.H_2O	70.6	0.15	6
With nitrogen			
Glycine	27	2	3
Serine	96.7	0.13	6
Brucine	96.5	0.6	6
Nicotinic acid	0.8	11	3
Nitromethane	6	10	3
Acetamide	2		6

The yields in Table I are all above 99% except for certain aromatics, in which a time effect was noted, indicating that oxidation may not be complete in these particular cases. On the other hand, results slightly under 100% are to be expected, considering the probable systematic errors. In the moisture and ash determinations traces of moisture (*7*) and nonoxidizable volatile material may have escaped detection. Volatile substances may be partially lost before oxidation can occur. Methanol, for example, gave variable results, 3 to 7% low, when oxidized as usual in a beaker, but gave essentially quantitative results when a reflux condenser (the only instance in Table I) was used.

Finally, results lower than theoretical may be caused by carbon monoxide formation, as noted by others. Christensen and coworkers (*2, 3*), using drastic conditions under which not only carbon monoxide but also oxygen is evolved, found it necessary to oxidize the carbon monoxide with a platinum heater to obtain quantitative results. Segal, Tripp, Tripp, and Conrad (*7*) found that approximately 1.0% of glucose and sucrose was converted to carbon monoxide under the conditions of their experiments, considerably more than under present conditions, although their results with sodium oxalate were almost identical with that in Table I.

The method was tested with known binary mixtures of pure substances (*1*). As models, mixtures of glucose and sucrose were blended in a series of proportions ranging from 5 to 95%. Upon analysis, the mean departure from the known values, or mean error, was 1.2%. The error is of course higher the smaller the difference between the equivalent weights of the two components, which for glucose and sucrose is only 5%. Two other series of binary mixtures, benzoic-phthalic acids and formic-oxalic acids, gave mean errors of 0.4 and 0.1%, respectively.

The types of substances listed in Table II resist oxidation by dichromate to various extents in the present method, and yields were not improved by use of prewarmed (60° C.) concentrated sulfuric acid. Methyl groups are slowly oxidized and in combination with carboxyl, as in acetic acid or acetamide, are almost quantitatively stable, although carboxyls in themselves, as in formic, oxalic, and malonic acids, are easily oxidized. If acetic

acid is one of the products, it may be sufficiently stable for a quantitative procedure. For example, the odor of acetic acid is easily recognized during the oxidation of rhamnose, a methyl carbohydrate, and calculation taking this into account gives a yield of 102.0%. Ethyl alcohol, also, is oxidized within a few per cent of theoretical to acetic acid.

Methylene groups are less stable than CH_3 groups, and their presence in propionic, succinic, and malic acids apparently causes these substances to be less stable than acetic acid. However, the effect of CH_2 groups depends entirely upon neighboring groups, for, whereas malonic and citric acids are quantitatively oxidized, malic and succinic acids are resistant to considerably differing degrees, as are lauric and stearic acids.

The presence of an OH group apparently renders a carbon atom quantitatively oxidizable, as in the carbohydrates and glycerol, tartaric acid, inositol, and perhaps, most strikingly, glycolic (hydroxyacetic) acid. The difficultly oxidizable CH_3 group in methanol is rendered quantitatively oxidizable by the OH group. In this respect the CH_3O group, and the ether linkage, —C—O—C—, are apparently in the same category as the OH group, as shown by the results with methyl mannoside, methyl galacturonide, methyl galacturonide methyl ester, and trimethyl methyl mannoside. In fact, it appears possible to generalize that the presence of an oxygen atom of any type, alcohol, carbonyl, carboxyl, ester, and ether renders the particular carbon atom to which it is attached completely oxidizable to carbon dioxide. This general effect of oxygen seems to arise from a specifically engendered oxidizability, rather than merely an oxidative opening wedge, inasmuch as nitromethane, with a partially oxidized carbon atom, is not completely oxidizable to carbon dioxide by the present technique.

The effect of amino nitrogen, itself quantitatively nonoxidizable (*2*, *3*), varies greatly. Glycine is more oxidized by the reagent than acetic acid; serine is almost but definitely less than completely oxidized; nicotinic acid, which resembles benzoic acid except that it has nitrogen in place of the meta carbon atom, is, however, practically nonoxidizable. In other positions the NH_2 group has little or no effect, as in acetamide or anthranilic acid. The +3 nitrogen of *p*-nitrophenol was quantitatively oxidized to the +5 state by the reagent.

The aromatic ring as well as unsaturated structures like malic acid are completely oxidized, although the yields for the aromatics tend to be somewhat lower than those of the aliphatics.

CONCLUSIONS

The results indicate that organic substances whose carbon atoms are all individually attached to oxygen atoms, or aromatics, can be determined with small error in the presence of moisture, ash, and other nonoxidizables, or as pure components in a binary mixture, with the method described, assuming complete oxidation of carbon to carbon dioxide. On the other hand, substances reproducibly oxidized to carbon dioxide plus a fairly stable intermediatelike acetic acid can be approximately quantitatively determined by taking this product into account theoretically, or accurately by standardizing the reagent against the pure substance.

For samples with one oxidizable component, *A*:

$$\% A = \frac{V \times N}{a} \times 100$$

For samples with pure components *A* and *B*:

$$\% A = \frac{V \times N - b}{a - b} \times 100$$

where V = volume in liters of dichromate solution of normality N reduced per gram of actual weight of sample, and a and b = equivalents of dichromate reduced per gram of pure components *A* and *B*. These values are either theoretical ones deduced from formula or empirical ones determined by experiment, in accordance with the results presented in this paper.

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Rapid Accurate Determination of Cellulose with the Dichromate Heat-of-Dilution Method

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The heat-of-dilution method for the determination of carbohydrates and certain types of other substances (*5*) is also applicable to celluloses. The α-cellulose determination is extensively used (*2*, *7*, *8*) in research and industrial control work on paper, rayon, wood pulps, and related products, and at least three standard (*1*, *10*) methods are in use in this country, whereas total cellulose is routinely determined in cotton (*3*). In most of these applications, speed and/or precision are essential, and many efforts have been made toward this end. This report presents comparable results with the heat-of-dilution method and another method requiring external heating, applied to identical samples of celluloses, starch, and glucose, with identical reagents.

METHOD

The heat-of-dilution method has been described (*5*). No attempt was made to dissolve the celluloses and precision oxidation was secured in the heterogeneous system. A 3-minute period after the addition of the acid was found to suffice for cellulose, starch, and glucose.

The method requiring external heating was essentially that used by Kettering and Conrad (*3*), in which 0.09 gram of cellulose, dry basis, is dissolved in 25 ml. of 12*M* sulfuric acid. Then 20 ml. of water and 10.00 ml. of 1.835*N* dichromate solution are added, and the mixture is boiled under reflux for 1 hour, and finally diluted and titrated as usual.

MATERIALS

The surgical cotton was a commercial product of unknown

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THE EFFECT OF SODIUM CITRATE ADMINISTRATION ON EXCRETION OF LEAD IN URINE AND FECES

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Previous experimental and clinical studies of sodium citrate administration in lead poisoning (1)(2)(3) demonstrated a highly significant reduction in blood lead concentration with rapid amelioration of symptoms during the administration of this drug. It is important to the proper understanding of the therapeutic action of sodium citrate to learn whether lead disappears due to (*a*) deposition in tissue and bones or (*b*) increased excretion. Preliminary studies of lead excretion in the urine gave variable results although a trend toward increased excretion was observed. Experiments to determine the fate of the lead which disappeared from the blood stream are reported in this paper. In these experiments total urinary and fecal lead excretion was measured before and during the administration of sodium citrate to nine individuals who were suffering from the effects of chronic exposure to lead.

EXPERIMENTAL. Patients selected for study had been exposed to industrial lead hazards for periods ranging from several months to several years. Eight of the nine patients exhibited definite evidence of lead poisoning. Clinical histories of these have been reported elsewhere (2). The remaining patient (R. R.), presented no definite signs of lead intoxication but had worked as a painter for many years.

After a preliminary period of hospitalization to eliminate effects of medication that some patients had received before admission, and to permit adjustment to hospital conditions, the collection of excreta was begun. All urine voided was collected in specially cleaned bottles containing 5 cc. of lead-free toluene as a preservative. Feces were collected in stainless steel bed pans and immediately transferred to waxed paper cartons. Precautions were taken throughout to avoid additions or losses of lead.

The experiments were divided into two stages: a control period, during which the patients received no medication known to influence lead metabolism, and a period of citrate therapy during which sodium citrate 5 grams three times daily was administered by mouth (a few patients received 20 grams daily). Throughout the study all patients received the standard house diet. In a few cases where constipation was present to a degree which might have interfered with the collection of feces, patients received 0.3 to 0.6 gram of cascara sagrada daily throughout the entire study.

Urine and feces were collected during 4 to 6 day periods and aliquot specimens were analyzed for lead by the method of Letonoff and Reinhold (4), slightly modified (5). Preparation of feces for sampling was facilitated by the addition of 5 cc. of lead-free hydrochloric acid to the total sample of feces and heating to dryness on a steam bath with frequent stirring. This procedure yielded a thoroughly mixed product from which representative samples were easily obtained after crushing in a mortar. The 24 hour excretion of lead in urine and feces for each period was calculated from these analyses.

RESULTS AND DISCUSSION. The results are presented graphically in figure 1. With one exception (J. W.) urinary lead excretion was increased during citrate

administration. Elimination in the feces and total output of lead were also increased during this period except in one case (J. N.). This patient had received citrate therapy during a 3 day period 4 days before the control period. The mean increase in total lead excretion during citrate administration was 0.652 ± 0.271 mgm. per 24 hours. This is statistically significant ($p = 0.05$ by Fisher's t Test (6)). Mean increases in daily urinary and fecal lead excretion of 0.108 ± 0.053 mgm. and 0.544 ± 0.251 mgm. respectively, during citrate therapy ($p = 0.08$ and 0.07) approach but do not quite attain statistical signif-

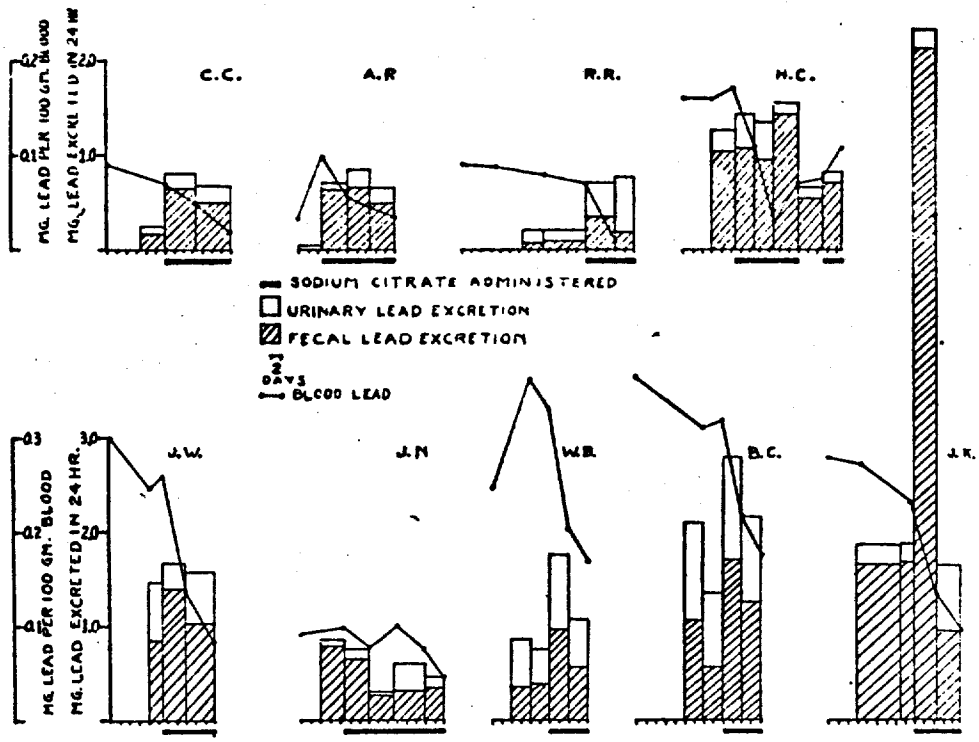

FIG. 1. CHANGES IN THE URINARY AND FECAL LEAD EXCRETION IN 9 PATIENTS WITH CHRONIC EXPOSURE TO LEAD, BEFORE AND DURING THE ADMINISTRATION OF SODIUM CITRATE

icance. While the increased lead excretion is mainly by way of the feces, a few patients show greater increase in urinary excretion. Greater individual variations explain the failure to demonstrate statistically significant increase in excretion in urine and feces considered separately, while the total excretion was significantly increased. After a marked initial increase in lead excretion during citrate administration, a downward trend in lead output is apparent. It was not possible to evaluate the duration of the action of citrate or the effects of subsequent courses of therapy because of unwillingness of our subjects to coöperate for longer periods. A statistically significant drop in blood lead was found coinci-

dent with the increased lead elimination. Blood lead figures have been reported elsewhere for 8 of the 9 patients (2).

No change in excretion of calcium or phosphorus in urine and feces was found during citrate administration in three subjects studied. The lead lost by increased excretion in feces and urine greatly exceeded the amount disappearing from the circulation as calculated from decrease in blood lead concentration.

It is of interest that Donnelly and Holman (7) have recently found sodium citrate to ameliorate the effects of uranium, a metal similar in many ways to lead in its action on the body.

SUMMARY

The excretion of lead in urine and feces of 9 patients after long-continued exposure to lead was determined before and during the administration of sodium citrate. Appreciable increases in urinary, fecal and total excretion in 8 of the 9 cases occurred during citrate therapy. The mean increase in total excretion of lead during citrate therapy for all 9 cases is statistically significant.

The authors are indebted to Dr. John G. Reinhold for helpful criticism and to the members of the nursing staff of the Medical Department for their conscientious coöoperation and invaluable assistance during this experiment.

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(5) Letonoff, T. V., Ind. and Eng. Chem., Anal. Ed., **13**: 631, 1941.
(6) Fisher, R. A., Statistical Methods for Research Workers, Edinburgh, 1936.
(7) Donnelly, G. L., and Holman, R. L., This Journal, **75**: 11, 1942.

of me ılts has little effect
zabl een pH 2 and 10.
a sc ccurs, above pH 10
K of the free acid is 5×10^{-5}.
a aq soln at pH 2.5 and may

ergents as a soil-suspending
rs, adhesives, printing inks,
and in general. In pharmacy

antacid. *Dose:* Oral as laxa-
of 5% soln.

mol wt 106.45. Na 21.60%,
lorate of commerce is about
ium chloride by electrolysis:
Chemicals, 2nd ed, (Wiley,

or white granules. d 2.5.
ates oxygen; entirely dec at
n 1 ml cold, 0.5 ml boiling
about 50 ml boiling alcohol,
diminishes its soly in water.
out of contact with organic
stances. LD orally in rats:

ssium chlorate, in manuf of
dyeing and printing fabrics;
s weed killer.
antiseptic wash.

common salt; the article of
ne salt, rock salt, or sea salt.
4%, Cl 60.66%. Occurs in
oduced by mining (rock salt),
derground salt deposits, and
ration: Faith, Keyes, Clark,
(Wiley, New York, 1957),
onograph: D. W. Kaufmann,
aph no. 145 (Reinhold, New

es, or powder; colorless and
in l crystals. d 2.17.
con ome calcium and
sorb ture and make it
atalize at a little above this
5 ml water at 25°, in 2.6 ml
very slightly sol in alcohol.
HCl and it is almost insol in
al. pH: 6.7–7.3. d of satd
aq soln of sodium chloride
D i.v. in rats: 2.5 g/kg.
e of chlorine and of sodium
ly all, their compds, *e.g.*,
dium carbonate, hydroxide,
uf soap, dyes—to salt them
dyeing and printing fabrics,
metallurgy of tin and other

eatment of salt depletion.
constipation. Topically (satd
Dose: i.v. as 0.9% soln,
ot generally considered poi-
. of NaCl for lactose in baby
ing.
ypertonic solns for irrigation
gestion of horses, cattle; as
soln to raise blood volume.
tomachic: horses and cattle:
urgative: horses: 125–500 g;

odium chloride contg trace
, copper, manganese, zinc

mol wt 90.45. Cl 39.20%,
on a commercial scale by
soln of sodium hydroxide
lime: Vincent, U.S. pats.
athieson). *Review* of manuf
aper Mag. Can. 53, no. 8,

or flakes, does not cake.
er, but will not explode on
with oxidizable material.

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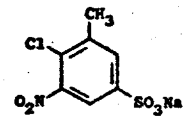
Solubility in water (g/100 g soln): At 5° = 34; at 17° = 39; at 30° = 46; at 45° = 53; at 60° = 55.
Trihydrate, $NaClO_2.3H_2O$, triclinic leaflets, becomes anhydr at 38° or in desiccator over KOH at room temp.
USE: In the prepn of chlorine dioxide for immediate use; in water purification; as bleaching agent for textiles, paper pulp.

Sodium 6-Chloro-5-nitrotoluene-3-sulfonate. *4-Chloro-5-nitro-m-toluenesulfonic acid sodium salt.* $C_7H_5ClNNaO_5S$; mol wt 273.64. C 30.72%, H 1.84%, Cl 12.96%, N 5.12%, Na 8.40%, O 29.24%, S 11.72%.



White or slightly yellow, cryst powder. Moderately sol in water, slightly in alcohol.
USE: As reagent for determination of potassium.

Sodium Chromate(VI). Neutral sodium chromate. $Na_2CrO_4.4H_2O$; mol wt 234.07. Anhydr salt 69.21%, H_2O 30.79%, Na 19.65%, CrO_3 42.73%, Cr 22.22%. For the anhydr: Na 28.38%, Cr 32.10%, O 39.51%.
It also crystallizes with $10H_2O$, mol wt 342.16; H_2O 52.65%, anhydr salt 47.35%, CrO_3 29.23%; but is unstable with respect to water content and melts at about 20°. Sodium chromate is also available in anhydr form as a powder.
Yellow, somewhat deliquesc crystals. Soluble in about 1 part water, slightly in alcohol. The aq soln is alkaline. *Keep well closed.* LD s.c. in rabbits: 243 mg/kg.
USE: Protection of iron against corrosion and rusting.

Sodium Chromate(VI), Radioactive. Rachromate; sodium radio-chromate (^{51}Cr); sodium chromate—^{51}Cr; $Na_2{}^{51}CrO_4$.
Sodium chromate is prepd from radioactive chromium (^{51}Cr) which has a half-life of 26.5 days. The emission of gamma rays is applicable to biological tagging and tracing. Other properties identical with those of ordinary sodium chromate. Available as soln for intravenous injection or for mixing with blood. Unbound chromate in the plasma can be reduced with ascorbic acid or may be removed by separation and washing of cells.
MED USE: Erythrocyte tracing agent.

Sodium Cinnamate. *Cinnamic acid sodium salt.* $C_6H_5CH{=}CHCOONa$; mol wt 170.14. $C_9H_7NaO_2$; C 63.53%, H 4.15%, Na 13.52%, O 18.81%; cinnamic acid 87.08%.
White, cryst powder. Soluble in 11 parts cold water; more sol in hot water and in water contg chlorides or nitrates; sol in about 160 parts alcohol; sol in glycerol.

* **Sodium Citrate.** Trisodium citrate; Citrosodine; Citnatin. $C_6H_5Na_3O_7.2H_2O$; mol wt 294.12. Anhydr salt 87.75%, H_2O 12.25%, anhydr citric acid 65.31%. For the anhydr: C 27.92%, H 1.95%, Na 26.73%, O 43.40%. It is at least 99% pure.
White, odorless crystals, granules or powder; cool, saline taste. Stable in air; becomes anhydr at 150°. Soluble in 1.3 parts water, 0.6 part boiling water; insol in alcohol. The aq soln is slightly alkaline to litmus. pH about 8. LD_{50} i.p. in rats: 1.76 g/kg.
Sodium citrate crystallizes also with $5H_2O$, in the form of relatively large, colorless crystals, or white granules. Anhydr salt 72.25%, H_2O 27.75%, anhydr citric acid 53.78%. It is not as stable as the dihydrate, drying out on exposure to air and also caking. *Keep well closed.*
USE: In photography; as sequestering agent to remove trace metals, to prevent coagulation of blood; in special cheeses.
MED USE: Systemic and urinary alkalizer, diuretic, expectorant, sudorific, in vitro anticoagulant. Has been used in lead poisoning, furunculosis. *Dose:* Oral, alkalizing 1 g.
VET USE: Anticoagulant for the collection of blood.

Sodium Citrate, Acid. Disodium citrate; disodium hydrogen citrate; Alkacitron. $C_6H_6Na_2O_7.1\frac{1}{2}H_2O$; mol wt 263.13. Anhydr salt 89.73%, H_2O 10.27%. For the anhydr: C 30.52%, H 2.56%, Na 19.48%, O 47.44%.
White powder, saline taste. One gram dissolves in slightly less than 2 ml water; pH of a 3% w/v soln in water 4.9 to 5.2. Used as an anticoagulant, generally in soln with glucose, to prevent the clotting of blood intended for transfusion. Preferable to sodium citrate, since it prevents caramelization of glucose on sterilization because of its acidity. A suitable soln contains 1.7 to 2%, and 2.5% dextrose; 120 ml of this soln prevents the clotting of 420 ml blood.

Sodium Cobaltinitrite. *Sodium hexanitrocobaltate(III).* $Na_3Co(NO_2)_6$; mol wt 403.98. Na 17.08%, Co 14.59%, N 20.81%, O 47.53%, NO_2 68.33%.
Yellow to brownish-yellow, cryst powder. Very sol in water, slightly in alcohol. Dec by mineral acids, but unaffected by dil acetic or similar organic acids. The aq soln dec gradually but if a few drops of acetic acid are added it may be kept for about 3 months.
USE: For the detection of potassium with which it forms a slightly sol compd.

Sodium Cresotate. $CH_3(OH)C_6H_3COONa$; mol wt 174.13. $C_8H_7NaO_3$; C 55.18%, H 4.05%, Na 13.21%, O 27.56%, cresotic acid 87.33%. It is usually a mixture of the salts of 2 or 3 isomeric cresotic acids.
White to reddish, microcryst powder; bitter taste. Soluble in water, alcohol.

Sodium Cyanide. Cyanogran. NaCN; mol wt 49.02. C 24.50%, N 28.58%, Na 46.92%, CN 53.08%. This cyanide of commerce is 95–98% pure. Mixtures of sodium cyanide with sodium chloride or carbonate for special uses are also marketed.
White granules or fused pieces. *Violent poison!* Odorless when perfectly dry; somewhat deliquesc in damp air and emits slight odor of HCN. mp 563°. Freely sol in water, slightly in alcohol. The aq soln is strongly alkaline and rapidly decomposes; the soln readily dissolves gold and silver in presence of air *Keep well closed.* MLD s.c. in rabbits: 2.2 mg/kg.
USE: Extracting gold and silver from ores; electroplating baths; fumigating citrus and other fruit trees, ships, railway cars, warehouses, etc.; manuf hydrocyanic acid and many other cyanides; case hardening of steel.
MED USE: 0.66% soln has been used to remove conjunctival stains. *Human Toxicity: See* Hydrogen Cyanide.

Sodium Diacetate. Sodium acid acetate; Dykon. $CH_3COONa.CH_3COOH$. Described as a "bound" compd of sodium acetate and acetic acid. Commercial development: Carbide & Carbon Chemicals Co.; Stein, Hall & Co., Inc., N.Y.
White powder, dec above 150°. Soluble in water, liberating 42.25% available acetic acid.
USE: Acetic acid in solid form; as an inhibitor of molds and rope-forming bacteria in bread: Glabe, *Food Inds.* 14, no. 2, 46 (1942).

Sodium Dichromate(VI). Sodium bichromate; bichromate of soda. $Na_2Cr_2O_7.2H_2O$; mol wt 298.05. Anhydr salt 87.91%, H_2O 12.09%, Na 15.43%, CrO_3 67.11%, Cr 34.90%. For the anhydr: Na 17.55%, Cr 39.70%, O 42.75%. Usually prepd from chromate according to the eq $2Na_2CrO_4 + H_2SO_4 \rightarrow Na_2Cr_2O_7 + Na_2SO_4 + H_2O$. Description of industrial processes: Müller, Glissmann in *Ullmann's Encyklopädie der technischen Chemie*, 3rd ed., vol. 5 (Munich, 1954), p 575.
Dihydrate, $Na_2Cr_2O_7.2H_2O$, reddish to bright orange, somewhat deliquescent crystals. Crystal system: monoclinic sphenoidal. Crystal habit: elongated prismatic. d_4^{25} 2.348. Bulk density: 96 lbs/cu ft. Becomes anhydr on prolonged heating at about 100°. The anhydr salt mp 356.7° and starts to dec at about 400°. Heat of soln −28.2 cal/g. Very sol in water. A satd aq soln contains: at 0° = 70.6% $Na_2Cr_2O_7.2H_2O$; at 20° = 73.18%; at 40° = 77.09%; at 60° = 82.04%; at 80° = 88.39%; at 100° = 91.43%. A 20% soln freezes at −3.5°, a 30% soln freezes at −6°, a 60% soln freezes at −26°, a 69% soln freezes at −48°. Specific heat of 20% soln at 25° = 0.85 cal/g/°C. Solns are acidic: pH of 1% soln = 4.0; pH of 10% soln = 3.5.
USE: Oxidizing agent in manuf of dyes, many other synthetic organic chemicals, inks, etc.; in chrome-tanning of hides; in electric batteries; bleaching fats, oils, resins, sponges; refining petroleum; manuf chromic acid, other chromates and chrome pigments; in corrosion-inhibitors, corrosion-inhibiting paints; in many metal treatments; electroengraving of copper; mordant in dyeing; for hardening gelatin; for the defoliation of cotton plants and other plants and shrubs, La Lande, U.S. pat. 2,760,854 (1956 to Pennsylvania Salt Mfg. Co.).

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eclampsia of dogs, cats. *Dose.* dogs: 0.3–4.0 g.

nate. *Lactobionic acid calcium ium bromide;* calcium galacto- icin; Calcibromin; Calcibronat. $_{12})_2Ca.CaBr_2$; mol wt 954.59. .20%, H 4.43%, Br 16.74%. pn: Isbell, *J. Research Natl. Bur.*

$_{12})_2Ca.CaBr_2.6H_2O$, hexagonal vgroscopic. Freely sol in water.

Carbamic acid calcium salt. 0.15. $C_2H_4CaN_2O_4$; C 15.00%, 7.49%, O 39.96%. Prepd from calcium hydroxide: Macleod, 334 (1905/6); Caro *et al.*, U.S.

Freely sol in water. Practically

alts of carbamic acid have low inan.

lenogen. CaC_2; mol wt 64.10. repn: Ehrlich in *Handbook of istry*, G. Brauer, Ed., (2nd ed, 3. *Review:* Brennan, *J. Electro-*

umps or orthorhombic crystals; of acetylene leaving a residue

gas for lighting purposes (1 kg acetylene); as reducing agent, opper sulfide to metallic copper; vice; manuf of calcium, iron, ; welding and cutting metals.

O_3; t 100.09. C 12.00%.

cipitated; precipitated chalk; mercial $CaCO_3$ produced by pure. The byproduct process, the calcium chloride process are outlined in *Kirk-Othmer's chnology*, vol. 4 (2nd ed, Inter- Sturge, Sturge, *Precipitated Manufacture and Standardiza-* ningham, England, 1948).

d; drop chalk; prepared chalk; white. Native $CaCO_3$ purified

or crystals. Two crystal forms ce: *Aragonite*, orthorhombic, at temps above 30°; *Calcite*, 1339° (102.5 atm), $d^{25.2}$ 2.711. Both forms occur in nature. O and CO_2. Practically insol *mpat:* Acids, alum, ammonium

er, plastics, paper, dentifrices, ticides, inks, shoe dressings; uticals, antibiotics; as a filler matches, pencils, crayons, welding rods; removing acidity or detecting and determining ions; with NH_4Cl for decom- aCl_2 soln for standardizing

ntidiarrheal. *Dose:* Oral 1 g. ration.

se: horses: 30–120 g; cattle: 5–15 g; dogs: 0.3–4.0 g; cats:

; mol wt 206.99. Ca 19.36%. Duveau, *Bull. Soc. Chim.* 10, t. 183,671 (1921).

, monoclinic hygroscopic n rapidly heated. Soluble in *K well closed.* LD_{50}

seed infectant. *Human* ate.

Calcium Chloride. $CaCl_2$; mol wt 110.99. Ca 36.11%, Cl 63.89%. Forms mono-, di-, tetra- and hexahydrates. Obtained as a byproduct in the ammonia-soda (Solvay) process and as a joint product from natural salt brines: Faith *et al.*, *Industrial Chemicals* (3rd ed, John Wiley & Sons, Inc., 1965), p 189.

Anhydrous, $CaCl_2$, cubic crystals, granules or fused masses. Very hygroscopic. mp 772°; bp >1600°; d_4^{15} 2.512. Freely sol in water (with liberation of much heat), alcohol. The commercial product is about 94–97% $CaCl_2$, the chief impurity being $Ca(OH)_2$. *Keep well closed.* LD_{50} orally in rats: 4.0 g/kg.

Dihydrate, $CaCl_2.2H_2O$, hygroscopic granules, flakes or powder. Apparent (bulk) density: 0.835. Freely sol in water, alcohol. The commercial grades contain 73–80% $CaCl_2$. *Keep well closed.*

Hexahydrate, $CaCl_2.6H_2O$, deliquesc trigonal crystals. mp 30°; d^{17} 1.68. Loses all H_2O at 200°. Extremely sol in water, alcohol. *Keep well closed.*

USE: The *anhydrous* form used as a drying and dehydrating agent for organic liquids and gases, and in desiccators. The *dihydrate* and *hexahydrate* forms are used for antifreeze and refrigerating solns, in fire extinguishers, etc. (a 40% soln freezes at −41°); to preserve wood, stone; manuf ice, glues, cements; fireproofing fabrics; automobile antifreeze mixtures; to melt ice and snow; as coagulant in rubber manuf, as size in admixture with starch paste; in concrete mixes to give quicker initial set and greater strength; freezeproofing of coal and ores; dust control on unpaved roads; sizing and finishing cotton fabrics; as brine for filling inflatable tires on tractors to increase traction.

MED USE: Electrolyte replacement. Has been used as diuretic, urinary acidifier, antiallergic. *Side Effects:* G.I., cardiac disturbances may occur.

VET USE: Dihydrate used like calcium gluconate. Also orally in ascites for dogs, cats. *Dose* (i.v. or orally): horses and cattle: 15–50 g; sheep and swine: 2–3 g (larger doses for larger hogs); dogs: 0.5–1.0 g.

Calcium Chromate(VI). C.I. 77223; C.I. Pigment Yellow 33; calcium chrome yellow; gelbin; yellow ultramarine. $CaCrO_4$; mol wt 156.09. Ca 25.68%, Cr 33.32%, O 41.00%. Prepn: Mylius, Wrochem, *Ber.* 33, 3689 (1900); Udy, U.S. pats. 2,493,789, 2,494,215 (both 1950); Dunn, O'Brien, U.S. pats. 2,745,764/5 (both 1956 to Vanadium Corp. of America). Also occurs as hemihydrate, monohydrate, and dihydrate.

Yellow monoclinic or rhombic crystals. Sparingly sol in water; sol in dil acids; practically insol in alcohol.

USE: As a pigment, corrosion inhibitor; manuf of chromium; in oxidizing reactions.

Calcium Citrate. $Ca_3(C_6H_5O_7)_2$; mol wt 498.44. $C_{12}H_{10}Ca_3O_{14}$; C 28.91%, H 2.02%, Ca 24.12%, O 44.94%. Prepn from citrus fruit: Cole, U.S. pat. 2,389,766 (1945 to California Fruit Growers Exchange). *Review:* Rudy, *Pharmazie* 4, 393 (1949).

Tetrahydrate, $Ca_3(C_6H_5O_7)_2.4H_2O$, odorless powder. Loses most of its water at 100° and all at 120°. Soluble in 1050 parts cold water; somewhat more sol in hot water; insol in alcohol.

USE: In the production of citric acid and other citrates; improvement of baking properties of flour.

Calcium Cyanamide. Calcium carbimide; "cyanamide"; nitrolime. N≡CN=Ca; mol wt 80.11. C 14.99%, Ca 50.03%, N 34.99%. Prepn: Kastens, McBurney, *Ind. Eng. Chem.* 43, 1020 (1951); Franck, Heimann, *Angew. Chem.* 44, 372 (1931); Owen, *Trans. Faraday Soc.* 57, 670 (1961); Dedman, Owen, *ibid.* 678.

Commercial grades may occur as grayish-black lumps of powder. While pure calcium cyanamide is nonvolatile and noncombustible, commercial grades may contain small amounts of calcium carbide which will produce acetylene in containers and processing vessels. Other contaminants are carbon, $Ca(OH)_2$, CaO, and $CaCO_3$. Pure calcium cyanamide occurs as glistening, hexagonal crystals belonging to the rhombohedral system. mp about 1200°; d_4^{20} 2.29. Tendency to sublime at 1150–1200°. Heat of formn from $CaC_2 + N_2 = -69.0$ kcal/mole (25°). Heat of fusion 1.29 cal/g. Essentially insol in water, but undergoes partial hydrolysis to the sol calcium hydrogen cyanamide, a source of cyanamide ions: $2CaNCN + 2H_2O \rightarrow Ca(HNCN)_2 + Ca(OH)_2$; $Ca(HNCN)_2 \rightarrow Ca^{++} + 2HNCN^-$. No known solvent will bring about soln without decompn.

USE: As fertilizer, defoliant, herbicide, pesticide; manuf and refining of iron; manuf of calcium cyanide, melamine, dicyandiamide. *Human Toxicity: See* Cyanamide.

VET USE: Anthelmintic for swine.

Calcium Cyanamide Citrated. Citrated calcium carbimide; CCC; carbimide; Dipsan; Abstem; Temposil. Contains citric acid in two parts by weight to one part of calcium cyanamide suitably purified for drug use. Formulations: de Grunigen, Ferguson, U.S. pat. 2,998,350 (1961 to Am. Cyanamid and to Alcoholism Res. Found., Toronto).

MED USE: Antialcoholic.

Calcium Cyanide. $Ca(CN)_2$; mol wt 92.12. C 26.08%, Ca 43.52%, N 30.41%. Prepn: *Gmelin's Handb. anorg. Chem.*, System no. 28 (Calcium), part B, 8th ed, pp 173–178, 958–960 (1958). The article of commerce contains 40–50% $Ca(CN)_2$.

Rhombohedric crystals or powder; dec in moist air liberating hydrogen cyanide. *Very poisonous!* Soluble in water with gradual liberation of HCN; even very weak acid (CO_2) liberates HCN; sol in alc. *Keep dry.*

USE: Fumigant; rodenticide; in stainless-steel manuf; stabilizer for cement. *Human Toxicity:* Qualitatively and quantitatively similar to HCN in proportion to CN content. *See also* Potassium Cyanide.

Calcium Cyclamate. *Cyclohexanesulfamic acid calcium salt;* calcium cyclohexanesulfamate; calcium cyclohexylsulfamate; cyclamate calcium; Cyclan; Sucaryl Calcium. $[C_6H_{11}NHSO_3^-]_2Ca^{++}$; mol wt 396.54. $C_{12}H_{24}CaN_2O_6S_2$; C 36.34%, H 6.10%, Ca 10.11%, N 7.07%, S 16.17%, O 24.21%. Prepn: Cummins, Johnson, U.S. pat. 2,799,700 and McQuaid, U.S. pat. 2,804,477 (both 1957 to Du Pont); Freifelder, U.S. pat. 3,082,247 (1963 to Abbott Labs.); Yamaguchi, Nakatsuchi, U.S. pat. 3,090,806 (1963 to Nitto Chemical Industry Co., Ltd. & Nitto Physico-Chemical Research Institute).

Obtained as the dihydrate, $C_{12}H_{24}CaN_2O_6S_2.2H_2O$. Crystals with pleasant, very sweet taste. Freely sol in water. Practically insol in alcohol, benzene, chloroform, ether. pH of 10% aq soln 5.5–7.5. Said to be more resistant to cooking temps than saccharin. *See* Cyclamic Acid.

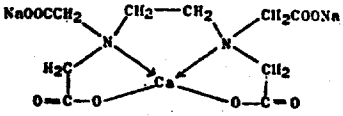
MED USE: Noncaloric sweetening agent.

Calcium Dichromate(VI). Calcium bichromate. $CaCr_2O_7$; mol wt 256.10. Ca 15.65%, Cr 40.62%, O 43.73%. Prepn: Hartford *et al.*, *J. Am. Chem. Soc.* 72, 3353 (1950).

Trihydrate, $CaCr_2O_7.3H_2O$, bipyramidal orange-red crystals. Nonhygroscopic if pure. Dec on heating above 100° to $CaCrO_4$ and CrO_3. d_4^{30} 2.370. Very sol in water; insol in ether, CCl_4, hydrocarbons; dissolves in alc with immediate reduction of the dichromate and pptn of brown hydrous chromic chromate; dissolves in acetone with subsequent pptn of $CaCrO_4$.

USE: As catalyst; in manuf of $CrCl_3$ and CrO_3; corrosion inhibitor.

Calcium Disodium Edetate. (*Ethylenedinitrilo*)*tetraacetic acid calcium disodium salt;* ethylenediaminetetraäcetic acid calcium disodium chelate; calcitetracemate disodium; calcium disodium (ethylenedinitrilo)tetraäcetate; calcium disodium ethylenediaminetetraäcetate; edathamil calcium disodium; edetic acid calcium disodium salt; Calcium Disodium Versenate; Mósatil; Antallin; Sormetal. $C_{10}H_{12}CaN_2Na_2O_8$; mol wt 374.28. C 32.09%, H 3.23%, Ca 10.71%, N 7.49%, Na 12.29%, O 34.20%. Prepn: Astakhov, Kiseleva, *Zhur. Obshch. Khim.* 20, 1780 (1950); *C.A.* 45, 2409 (1951).



Tetrahydrate, $Na_2(CaN_2C_{10}H_{12}O_8).4H_2O$, powder. Soluble in water: at 30° an 0.1 molar soln can be prepd. Practically insol in organic solvents. pH of 0.1 molar aq soln about 7. Exchanges its calcium for lead or other heavy metal ions forming water-soluble complexes of the heavy metals.

MED USE: Chelating agent; detoxicant for poisoning by lead and other heavy metals. *Dose:* Oral (not recommended for acute poisoning) 4 g/day; i.v. (for acute lead poisoning)

. O 47.00%, SO_4 70.55%.

es, or granules. d 2.24.
water and is converted into
rts water, 0.85 part boiling

s and siliceous compds.
ime laxative.

m hydrosulfide; potassium
fhydrate. KHS; mol wt
4.42%. Prepd industrially
e, Ger. pat. 380,385 (1922);
S. pat. 1,662,735 (1925);
,771,384 (1926 to Dow).
ion of dry H_2S upon potas-
ol: Rule, *J. Chem. Soc.* 99,
88, 102 (1934).
s or white, strongly hygro-
resent as the hemihydrate,
d 1.70. Rapidly becomes
mation of polysulfides and
0°. mp 450–510° forming a
ion: +62.5 kcal. Heat of
HS.$\frac{1}{2}H_2O$ at 16° = +0.62

ssium acid tartrate; acid
ydrogen tartrate; cream of
faecla. $KHC_4H_4O_6$; mol
%, H 2.68%, K 20.78%,
. Obtained from the sedi-
own as argols or wine lees.
See also Argol and Tartaric

st powder; pleasant acidu-
in 162 ml water, in 16 ml
: readily sol in dil mineral
x. Solubility in water also
bout 100°.
s; g metals, galvanic
O_3 in mordants for wool.
hartic.
animals, diuretic for large
ses and cattle: 15–45 g;
: 4–15 g.

Potassium tetrahydroborate.
%, B 20.06%, H 7.47%.
noxyborohydride and dibo-
o the eq $3KB(OCH_3)_4$ +
: Schlesinger *et al., J. Am.*
mercial process from sodium
: Banus, Bragdon, Hinck-
1954).
difference from $NaBH_4$).
bout 500° without melting.
ally more stable and less
H_4. Negative heat of soln
bility (w/w) in water at
= 20%; ethylenediamine
0° = 0.7%; dimethylform-
ny in a 4:1 water–methanol
aq solns are stable.
de. *Human Toxicity:* No
ydrides are quite toxic and
, CNS.

assium tartratoborate; sol-
ream of tartar; potassium
evaporating a soln of 2
a bitartrate.
y sol in water.
ography as a retarder for

mol wt 167.01. K 23.41%,

27. mp about 350°, decom-
ion of oxygen. Soluble in
g water; almost insol in

g a anal. chemistry.
caus vomiting, diarrhea,

254/a

Potassium Bromide. KBr; mol wt 119.01. K 32.85%, Br 67.15%. Contains at least 99% KBr. Continuous electrolytic process of prepn: Maylott, Elkins, U.S. pat. 2,989,450 (1961 to Dow Chem. Co.).

Colorless crystals or white granules or powder. d 2.75. mp 730°. One gram dissolves in 1.5 ml water, 1 ml boiling water, 250 ml alcohol, 21 ml boiling alcohol, 4.6 ml glycerol. The aq soln is neutral.

USE: Manuf photographic papers and plates; process engraving.

MED USE: Sedative, antiepileptic. *Dose:* Oral 0.3 to 1 g. *Side Effects:* Rash, bromism may occur. *Human Toxicity:* Large doses cause CNS depression. Prolonged intake may cause mental deterioration, acneform skin eruptions.

VET USE: In epileptiform fits, chorea, puerperal eclampsia, early pericarditis of dogs; esophagismus of horses. *Dose:* horses and cattle: 25–50 g; sheep and swine: 2–5 g; dogs: 0.3–2.0 g.

Potassium Carbacrylic Resin. A generic name for the potassium form of cross-linked polyacrylic polycarboxylic ion exchange resins.

Potassium Carbonate. Salt of tartar; pearl ash. K_2CO_3; mol wt 138.20. C 8.69%, K 56.58%, O 34.73%.

Hygroscopic, odorless granules or granular powder. d 2.29; mp 891°. Soluble in 1 part cold, 0.7 part boiling water; practically insol in alcohol. Its aq soln is strongly alkaline. pH 11.6. *Keep tightly closed.*

Sesquihydrate, $K_2CO_3.1\frac{1}{2}H_2O$, small granular crystals. When it contains the full amount of water (16.36%) it is not hygroscopic. Soluble in less than 1 part water; practically insol in alcohol. The aq soln is strongly alkaline.

USE: Manuf soap, glass, pottery, smalts and many potassium salts; in process engraving and lithography; tanning and finishing leather; liq shampoos; for removal of water from organic liqs; in anal. chemistry.

MED USE: Formerly as systemic alkalizer, diuretic. *Human Toxicity:* Irritant and caustic with action similar to, but less severe than, potassium hydroxide, *q.v.*

Potassium Chlorate. Potcrate. $KClO_3$; mol wt 122.55. K 31.91%, Cl 28.93%, O 39.17%. Contains at least 99% $KClO_3$.

Colorless, lustrous crystals, or white granules or powder. d 2.32. mp 368°; above this temp it dec into perchlorate and oxygen. One gram dissolves slowly in 16.5 ml water, 1.8 ml boiling water, about 50 ml glycerol; almost insol in alcohol. *Keep out of contact with organic matter or other oxidizable substances. Caution:* Explodes with sulfuric acid; inflames with explosion if triturated with any organic substances, sulfur, phosphorus, sulfite, hypophosphite, and other oxidizable substances. *Incompat.* Iodides, tartaric acid.

USE: Explosives; fireworks; matches; printing and dyeing cotton and wool black; manuf aniline black and other dyes; source of oxygen; in chemical analyses.

MED USE: Formerly as feeble antiseptic for skin, mucous membranes. Also in hyperthyroidism, thyrotoxicosis. *Human Toxicity:* Irritating to G.I. tract, kidney. Can cause hemolysis of red blood cells and methemoglobinemia. Toxic dose approx 5 g. Combined iodine and perchlorate is not recommended. Cough remedies which contain iodide are to be avoided.

VET USE: A 2 to 3% soln is a useful mouthwash for stomatitis. Similar solns are also used in vaginitis.

Potassium Chloride. Kaleorid; Kalitabs; Potavescent; Rekawan; Slow-K Tablets; Span-K. KCl; mol wt 74.55. K 52.44%, Cl 47.56%. Contains at least 99% KCl. Occurs in nature as the mineral *sylvine* (*sylvite*).

White crystals or cryst powder. d 1.98. mp 773°. One gram dissolves in 2.8 ml water, 1.8 ml boiling water, 14 ml glycerol, about 250 ml alcohol; insol in ether, acetone. Hydrochloric acid, sodium or magnesium chlorides diminish its soly in water. d of saturated aq soln at 15° is 1.172. pH: about 7.

USE: In photography; in buffer solns, electrode cells.

MED USE: Prophylaxis and treatment of hypokalemia, digitalis intoxication. *Side Effects:* Small intestine ulceration may occur with oral administration. *Human Toxicity:* Large doses by mouth can cause G.I. irritation, purging, also weakness and circulatory disturbances.

Potassium Chromate(VI). Neutral potassium chromate. K_2CrO_4; mol wt 194.20. K 40.26%, CrO_3 51.50%, Cr 26.78%, O 32.96%.

Lemon-yellow crystals; d 2.73; mp 975°. Soluble in 1.6 parts cold, 1.2 parts boiling water; insol in alcohol. The aq soln is alkaline to litmus or phenolphthalein. LD s.c. in rabbits: 12 mg/kg.

USE: Has a limited application in enamels, finishing leather, rustproofing of metals, being replaced by the sodium salt; as reagent in anal. chemistry. *Human Toxicity: See* Chromium Trioxide.

Potassium Citrate. $K_3C_6H_5O_7.H_2O$; mol wt 324.34. Anhydr salt 94.47%, H_2O 5.53%. For the anhydr: C 23.52%, H 1.64%, O 36.55%, K 38.28%. It is at least 99% pure.

White crystals, granules or powder. Loses its water at 180°. One gram dissolves in 0.65 ml in water; very slowly in 2.5 ml glycerol; almost insol in alcohol. The aq soln is alkaline to litmus; pH about 8.5.

MED USE: Prophylaxis and treatment of hypokalemia; expectorant, sudorific, diuretic, urinary alkalizer, gastric antacid. *Dose:* Oral 1 to 2 g.

VET USE: As a nonirritating diuretic. *Dose:* horses: 25–50 g; cattle: 50–100 g; dogs: 0.5–2.0 g; cats: 0.1–0.3 g.

Potassium Citrate, Monobasic. Monopotassium citrate. $KC_6H_7O_7$; mol wt 230.21. C 31.30%, H 3.07%, O 48.65%, K 16.98%, anhydr citric acid 83.46%.

White, cryst powder. Soluble in water; the soln is subject to molding.

Potassium Cobaltous Selenate. $K_2Co(SeO_4)_2.6H_2O$; mol wt 531.15. K 14.72%, Co 11.10%, Se 29.73%, O 42.17%, H 2.28%, SeO_3 47.81%. Anhydr form 79.65%, H_2O 20.35%. Prepd by evaporating a soln of the component salts: von Hauer, *Sitzungsber. Akad. Wien* 39, 839 (1860).

Garnet-red monoclinic crystals. d 2.514. Stable in air.

Potassium Cyanate. KCNO; mol wt 81.11. C 14.81%, N 17.27%, K 48.20%, O 19.72%, CN 32.08%.

White, cryst powder. d 2.05. Soluble in water, very slightly in alcohol.

Human Toxicity: Slowly converted in body to cyanide. *See* Hydrogen Cyanide.

Potassium Cyanide. KCN; mol wt 65.11. C 18.44%, K 60.05%, N 21.51%, CN 39.95%. The article of commerce contains about 95% KCN.

White, deliquesc, granular powder or fused pieces; odor of HCN. *Violent poison!* On exposure to air it is gradually dec by CO_2 and moisture. d 1.52; mp 634°. Soluble in 2 parts cold, 1 part boiling water, 2 parts glycerol, 100 parts alcohol, 25 parts methanol. The aq soln is strongly alkaline and rapidly dec. pH of 0.1*N* aq soln = 11.0. *Keep tightly closed and protected from light.* MLD i.v. in rats: 2.5 mg/kg; LD orally in dogs: 1.6 mg/kg. *Incompat.* Acids and acid syrups; alkaloids, chloral hydrate, iodine, metallic salts, permanganates, chlorates, peroxides.

USE: Similar to, but is largely replaced by, sodium cyanide.

MED USE: Formerly as soln instilled into conjunctival sac for removal of silver nitrate stains. *Human Toxicity:* Poisoning may occur by ingestion, absorption through injured skin or inhalation of hydrogen cyanide liberated by action of carbon dioxide or other acids. Strong solns are corrosive to skin. For symptoms *see* Hydrogen Cyanide.

Potassium Dichromate(VI). Potassium bichromate. $K_2Cr_2O_7$; mol wt 294.21. Cr 35.36%, K 26.58%, CrO_3 67.98%, O 38.07%. In the U.S.A. it is usually prepd by the reaction of potassium chloride on sodium dichromate: $Na_2Cr_2O_7 + 2KCl \rightarrow K_2Cr_2O_7 + 2NaCl$. *Ref:* Vetter in Kirk-Othmer, *Encyclopedia of Chemical Technology*, vol. 3 (New York, 1949), p 951. In Germany it is obtained from potassium chromate produced by roasting the chrome ore with KOH. *Ref:* Müller, Glissmann in *Ullmann's Encyklopädie der technischen Chemie*, 3rd ed, vol. 5 (Munich, 1954), p 580.

Bright orange-red crystals. Not hygroscopic or deliquescent (difference from sodium dichromate). Crystal habit: prismatic. Crystal system: triclinic pinacoidal, transition to monoclinic at 241.6°. d_4^{25} 2.676. Bulk density: 100 lbs/cu ft. mp 398°. Dec at about 500°. Heat of fusion 29.8 cal/g. Heat of soln −62.5 cal/g. Specific heat 0.186 at 16°–98°. Soluble in water. A satd aq soln contains at 0° = 4.3%, at 20° = 11.7%, at 40° = 20.9%, at 60° = 31.3%, at 80° = 42.0%, at 100° = 50.2%. Acid reaction: A 1% aq soln has a pH of 4.04 and a 10% soln has a pH of 3.57.

USE: In tanning leather, dyeing, painting, decorating porcelain, printing, photolithography, pigment-prints, stain-

254a

rum· erfumery; the alcoholic soln
d "pi ole oil."

oate. $CH_3(CH_2)_4COOC_2H_5$;
O_2. 71.95%, H 12.08%, O 15.97%.
acid 86.00%.
62. bp 243–245°. Insoluble in water;
hloroform, ether.
nquets, cognac essence.

thyl hexanoate. $CH_3(CH_2)_4COO$-
$C_8H_{16}O_2$; C 66.63%, H 11.18%,
5%, caproic acid 80.55%.
sh liq; pleasant odor. d^{20} 0.873.
e in water; miscible with alcohol,

fruit flavors.

yl octanoate; ethyl octylate. CH_3-
wt 172.26. $C_{10}H_{20}O_2$; C 69.72%,
cohol 26.74%, caprylic acid 83.72%.
obile liq; pleasant, pineapple odor.
. Insoluble in water; miscible with

rs; constit of enanthic, cocoic, and

arbonic acid diethyl ester; diethyl
$_5O)_2C{=}O$; mol wt 118.30. C 5-
53%, O 40.63%. Prepn: Palomaa
Manuf: Mador, Blackham, U.S.
National Distillers & Chemical

easant ethereal odor. mp −43°.
n_D^{20} 1.3843. Practically insol in
hol, ether.
ellulose; manuf radio tubes; fixing
ments.

ethyl ether of cellulose; Ethocel.
or chemical cotton by treatment
of the alkali cellulose with ethyl
bliography: E. Ott, *Cellulose and*
ed (New York, 1955).
solub is dependent upon the
om ethyl cellulose has an
A % product softens at 140°
s, ethylene dichloride, benzene,
ate, acetone, methanol, ethanol,
hloride.
lastics and lacquers. To avoid
formulations usually include an
hoquinone monobenzyl ether, 4-
nylamine.

Chaulmestrol; Moogrol. Essen-
yl ester of the unsaturated fatty
ydnocarpic) of chaulmoogra oil.
ight fruity odor; not unpleasant
d_{25}^{25} about 0.904. $[\alpha]_D^{25}$ +44.5°
n water; miscible with alcohol,
35 ml/kg.
nosis. *Dose:* Oral, i.m. 0.3–5 ml.
rritation may occur.

thane; monochlorethane; chlor-
ether chloratus; ether hydro-
ne; Chelen; Anodynon; Chloryl
$_5Cl$; mol wt 64.52. C 37.23%,
Cl 56.53%; ethanol 71.40%.
ne on ethylene in the presence
2,393,509 (1946); by the action
ne presence of the chlorides of
d calcium: Ger. pat. 298,931;
em. 1930, 233, 286; by heating
S. pat. 2,396,639 (1946); *C.A.*

y temp and pressure. Char-
ing taste. At low temps or
nyl chloride is a mobile, very
apor density: 2.22 (air = 1.00).
When ethyl chloride is liberated
its sealed container (usually a
it vaporizes at once. Boiling
han one atm: 2 atm = 32.5°;
5 atm = 64.0°; 10 atm = 92.6°; 20 atm = 127.3°; 30 atm = 149.5°; 40 atm = 167.0°; 50 atm = 180.5°. Critical temp = 187.2°; critical press = 52.0 atm. Slightly sol in water: 0.574 g/100 ml at 20°. Soluble in alcohol: 48.3 grams dissolves in 100 ml alcohol. Miscible with ether. Ethyl chloride burns with a smoky, greenish flame, producing hydrogen chloride. Flash pt: −50° (−58°F) (closed cup); −43° (−45°F.) (open cup). Explosive limits (% by vol in air): lower 3.6; upper 14.8. *Caution:* Keep away from heat, sparks, and open flame. Keep container closed, out of sun, and away from heat. Use with adequate ventilation. Carbon dioxide is recommended for extinguishing ethyl chloride fires.

USE: Refrigerant, solvent, alkylating agent, starting point in the manuf of tetraethyl lead: U.S. pat. 1,907,701.

MED USE: Topical anesthetic (by freezing). Has been used also for inhalation anesthesia. *Human Toxicity:* Mildly irritating to mucous membranes. High concns of vapors cause narcosis, unconsciousness.

VET USE: Local anesthetic for minor surgical procedures in cats, birds and other small animals.

Ethyl Chloroacetate. $CH_2ClCOOC_2H_5$; mol wt 122.55. $C_4H_7ClO_2$. C 39.20%, H 5.76%, Cl 28.93%, O 26.11%, acetic acid 49.00%, chloroacetic acid 77.11%.

Liquid; pungent odor. *Vapors irritate the eyes.* d_4^{20} 1.159. Solidif −26°. bp 144–146°. Flash pt 54°. n_D^{20} 1.4227. Insoluble in water; miscible with alcohol, ether.

Ethyl Chloroformate. *Chloroformic acid ethyl ester;* ethyl chlorocarbonate. $ClCOOC_2H_5$; mol wt 108.53. $C_3H_5ClO_2$; C 33.20%, H 4.64%, Cl 32.69%, O 29.48%. Prepn from phosgene and alc: Dumas, *Ann.* 10, 277 (1834).

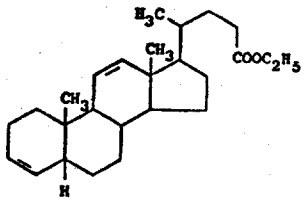
Liquid; vapors strongly irritate eyes and mucous membranes. Flammable. Flash pt slightly below 61°F. bp 95°. d_4^{20} 1.1403. n_D^{20} 1.3947. Practically insol and gradually dec by water; miscible with alc, benzene, chloroform, ether.

Human Toxicity: Irritant and corrosive to skin, mucous membranes.

Ethyl α-Chloropropionate. $CH_3CHClCOOC_2H_5$; mol wt 136.58. $C_5H_9ClO_2$; C 43.97%, H 6.64%, Cl 25.96%, O 23.43%, alcohol 33.73%, chloropropionic acid 79.49%, propionic acid 54.24%.

Liquid; pleasant odor. d_4^{20} 1.087. bp 147–148°. n_D^{20} 1.4185. Insoluble in water; miscible with alcohol, ether.

Ethyl Choladienate. Choladienic acid ethyl ester. $C_{26}H_{40}O_2$; mol wt 384.58. C 81.20%, H 10.48%, O 8.32%. Usually a mixture of $\Delta^{2,11}$ and $\Delta^{3,11}$-choladienic acid ethyl esters. Prepn from lithocholic acid: Wieland *et al.*, *Z. Physiol. Chem.* 241, 47 (1936); from Δ^{11}-lithocholenic acid: Bernstein, Querry, U.S. pat. 2,725,388 (1955 to American Cyanamid). The structure shown here is $\Delta^{3,11}$.



Crystals from methanol, mp 68–70°. Soluble in methanol, chloroform.

Ethyl Cinnamate. Ethyl phenylacrylate. $C_6H_5CH{=}CH$-$COOC_2H_5$; mol wt 176.21. $C_{11}H_{12}O_2$; C 74.99%, H 6.86%, O 18.16%, alcohol 26.14%, cinnamic acid 84.08%.

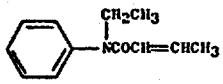
Almost colorless, oily liq; faint cinnamon odor. d_4^{20} 1.049. bp 271°. mp 6–10°. n_D^{20} 1.5598. Insoluble in water; miscible with alcohol, ether.

USE: As fixative for perfumes; in glass prisms, lenses.

* **Ethyl Citrate.** Triethyl citrate. $C_6H_5O_7(C_2H_5)_3$; mol wt 276.28. $C_{12}H_{20}O_7$; C 52.16%, H 7.30%, O 40.54%, alcohol 50.04%, anhydr citric acid 69.54%.

Bitter, oily liq. d^{20} 1.137. bp_{760} 294°; $bp_{1.0}$ 127°. Viscosity at 25° = 35.2 centipoises. Pour point about 10°. n_D^{20} 1.4455. Solubility in water about 6.9%, soly in peanut oil 0.8%. Miscible with alcohol, ether.

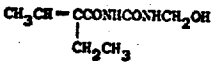
***N*-Ethylcrotonanilide.** *N*-Ethyl-*N*-phenylcrotonamide; *N*-crotonoyl-*N*-ethylaniline; *N*-ethyl-*N*-phenylethylidenacetamide; crotonic acid *N*-ethylanilide. $C_{12}H_{15}NO$; mol wt 189.25. C 76.15%, H 7.99%, N 7.40%, O 8.45%. Prepn: Britton *et al.*, U.S. pat. 2,368,195 (1945 to Dow Chem. Co.).



Crystals, mp 45–47°. bp_{12} 142–144°.

MED USE: Antipruritic.

***N*-2-Ethylcrotonyl-*N'*-methylolurea.** $C_8H_{14}N_2O_3$; mol wt 186.21. C 51.60%, H 7.58%, N 15.04%, O 25.78%. Prepd by refluxing carbromal with silver oxide in isopropanol and treating the resulting 2-ethylcrotonylurea with akaline formaldehyde soln: Fancher, U.S. pat. 2,734,082 (1956 to Miles Labs., Inc.).

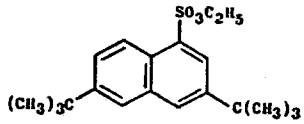


Hydrated crystals from ethanol, shrinking at 124–126°, fusing with effervescence at 165–175°. Moderately sol in water, alcohol, other organic solvents.

Ethyl Cyanoacetate. Cyanoacetic ester; ethyl cyanoethanoate; the ethyl ester of cyanoacetic acid; malonic acid ethyl ester nitrile. $CNCH_2COOC_2H_5$; mol wt 113.12. $C_5H_7NO_2$; C 53.09%, H 6.24%, O 28.29%, N 12.39%, CN 23.0%. Made by the interaction of sodium cyanide and chloroacetic acid and subsequent esterification of the cyanoacetic acid formed: Kohler, Allen, *Org. Syn.* 3, 53 (1923); Inglis, *ibid.* 8, 74 (1928).

Liquid. Slight, pleasant odor. d_4^{25} 1.0560; d_4^{50} 1.0306; d_4^{70} 1.0110. mp −22°. bp_{760} 206.0°; bp_{100} 152.8°; bp_{40} 133.8°; bp_{20} 119.8°; bp_{10} 106.0°; bp_5 93.5°; $bp_{1.0}$ 67.8°. $n_D^{20.5}$ 1.41793. Insoluble in water; miscible with alcohol, ether; sol in ammonia water, aq solns of alkalies.

Ethyl Dibunate. *3,6-Di-tert-butyl-1-naphthalenesulfonic acid ethyl ester;* ethyl 3,6-di-*tert*-butyl-1-naphthalenesulfonate; 2,7-di-*tert*-butylnaphthalene-4-sulfonic acid ethyl ester; ethyl 2,7-di-*tert*-butylnaphthalene-4-sulfonate; dibunate ethyl. $C_{20}H_{28}O_3S$; mol wt 348.51. C 68.93%, H 8.10%, O 13.77%, S 9.20%. Prepd from 3,6-di-*tert*-butyl-1-naphthalenesulfonyl chloride and ethanol: Menard *et al.*, *Can. J. Chem.* 39, 729 (1961).



Crystals from ethanol, mp 138–139°.

MED USE: Antitussive.

Ethyl Dichloroacetate. $CHCl_2COOC_2H_5$; mol wt 157.00. $C_4H_6Cl_2O_2$; C 30.60%, H 3.85%, O 20.38%, Cl 45.17%, alcohol 29.34%, dichloroacetic acid 82.14%, acetic acid 38.25%.

Colorless liq. d_4^{20} 1.282. bp 158°. n_D^{20} 1.4386. Slightly sol in water; miscible with alcohol, ether.

Ethyl Diethylmalonate. $C_2H_5COOC(C_2H_5)_2COOC_2H_5$; mol wt 216.27. $C_{11}H_{20}O_4$; C 61.09%, H 9.32%, O 29.59%, alcohol 42.60%, diethylmalonic acid 74.06%, malonic acid 48.12%. Produced by the action of sodium ethylate and ethylbromide on diethyl malonate.

Liquid. d 0.985–0.990. bp 228–230°. n_D^{20} 1.424. Insoluble in water; miscible with alcohol, ether.

USE: Manuf barbiturates.

5-Ethyldihydro-6-phenyl-2*H*-1,3-thiazine-2,4-(3*H*)-dione. 5-Ethyl-6-phenyl-*meta*-thiazane-2,4-dione; 2,4-dioxo-5-ethyl-6-phenyltetrahydro-1,3-thiazine; MRD-125; Dolitrone. $C_{12}H_{13}NO_2S$; mol wt 235.31. C 61.25%, H 5.57%, N 5.95%, O 13.60%, S 13.63%. Prepn: Wheeler, Gash, U.S. pat. 2,585,064 (1952 to Merrell); Taborsky, *J. Org. Chem.* 23, 1779 (1958).

On the question of safety of citrate blood transfusion

Experimental investigations of the toxic and lethal dose of sodium citrate, as well as of its influence of the serum calcium level and on the thrombocytes

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The transfusion of sodium citrate blood is possible without any major apparatus. If the sodium citrate method is nevertheless not very popular in Germany - in contrast to USA and France - this is due to the widely held view that sodium citrate blood is no longer standard blood and that the sodium citrate incorporated with the blood is harmful to the receiver. But most authors fail to substantiate this view (e.g. G.H. Schneider, W. Baer, P. Schumacher et al.)

The necessity of effecting a blood transfusion rapidly is much rarer in internal medicine and in surgery than it is in obstetrics. The obstetrician (we also include among them the gynecologist who operates on a burst extrauterine pregnancy) has no time to lose in a strict indication and he requires a method which is absolutely safe and which permits a rapid transfusion. The method should permit any surgically trained physician to carry it out with any special skill and without coupling the donor with the receiver, and finally to combine it with an ordinary saline infusion. The rapid success of a blood transfusion in obstetrics depends on the fulfillment of these requirements.

In a strict indication a blood transfusion is rarely necessary, even with a large number of obstetric-gynecological patients. It is therefore impossible that a great number of physicians will master completely the technique of a complicated transfusion apparatus (Gehlecker, Percey, Beck et at.), so that a skilled physician is always available. But each transfusion apparatus has its bugs, as it is known from many studies. Furthermore, with strict selection of the cases, we find that an intravenous saline infusion is

carried out much more frequently to replace the blood. Not so rarely a blood transfusion becomes only necessary during the further clinical source. Finally the use of all transfusion apparatus where donor and receiver must be closely coupled with each other, has the great disadvantage that operations which must be carried out simultaneously are greatly hindered. If the patient has to be placed during the blood transfusion from the bed on the operating table or be repositioned on the operating table (for example, change from vaginal operation to abdominal operation) a direct blood transfusion must be interrupted; The simultaneous repositioning of the donor presents difficulties, it may even be impossible occassionly, because of the space conditions. These disadvantages, which are only outlined here briefly, are eliminated in a citrate blood transfusion. It starts with an intravenous saline infusion; the transfusion fluid is obtained and prepared in an adjoining room, and the donor is spared the sight of a fight for a human life. By filling the citrate blood into the salt burette, the saline infusion is transformed into a blood transfusion whose size can be varied as required. Finally any repositioning of the patient is easily possible despite the saline infusion.

Characteristic of the practical needs in obstetrics is the fact that two leading obstetricians, Bumm and Doederlein, have warly advocated citrate blood transfusions. The Stoeckel Clinic has used it for years, after many tests with different transfusion apparatus showed unsatisfactory results.

Fig. 1 Apparatus for citrate blood transfusion at the Stoeckel Clinic

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Fig. 1 shows the apparatus which is used at the Stoeckel Clinic in a citrate blood transfusion. Two marks are made on a 100 cc graduated cylinder with thin leucoplast tapes. As it can be seen from the label, the graduated cylinder is to be filled up to the first mark with 25 cc 5% sodium citrate and 250 cc normosal solution. Since 25 cc are already measured in the sodium citrate bottles, this amount of liquid is first poured into the graduated

cylinder, then normosal solution is added up to the first mark; the exact amount of 250 cc has thus been reached.

After puncturing the vena cubitalis in the donor, the blood is transferred into the graduated cylinder through the puncturing canule until the liquid amount has reached the second mark. Exactly 250 cc donor blood have thus been collected and a 0.25% sodium citrate blood solution is prepared. If somewhat more blood should have passed over accidentally, this does not matter. The sodium citrate concentration is so high that there is no coagulation if the mixing ratio is slightly exceeded. The excess blood can be easily read on the graduation of the cylinder. The citrate blood mixture is not stirred, only shaken slightly in the graduated cylinder.

We think that the number of those who use the citrate method in blood transfusion will grow in Germany too, just as in USA, where the Mayo Clinic, for example, can look back on thousands of citrate blood transfusions. A prerequisite, however, is the assurance that the citrate blood does not cause any major disturbances. In order to prove this, it is necessary first to answer the question whether cases are known where patients died after blood transfusions or re-infusions which are attributable exclusively to the addition of sodium citrate to the transfused blood, and second to test in the animal experiment how the test object behaves after the incorporation of sodium citrate.

We have tried to compile from the literatures as accurately as possible fatal cases after blood transfusions. Two statistics are already available: a study by P.Schneider of the year 1925, who reported about 25 deaths in the last 4 years, and a compilation of deaths from transfusion in the Breitner monography. Schneider could show that all the deaths reported by him are true to incompatibility of the blood group of donor and receiver. Never where the donors so selected that they belonged to the same blood group as to the receivers. Either no test for agglutination was made at all, or a mistake was made in the test; or only a test for mutual agglutination was

made(major mutual agglutination test or Nuernberg test) which does not positively exclude isoagglutinins according to present views. The cause of death in the cases checked by Schneider was hemolysis, except for the case described by Carrington, Lunsford and Leo, where death occurred under the syndrome of an anaphylactic shock. In the cases collected by Breitner (18 authors) it could likewise be shown that death was due to imcompatibility of the blood types. To these statistical cases by Schneider and Breitner we can add 18 other cases described in the literature, among others a few deaths after re-infusion.

In 7 cases a direct blood transfusion was carried out with the Gehlecker- or Percey apparatus (2 cases by Schumacher, 1 case each by Denk, Haebler Clairmont Metzler and Dsuesstrunk); these cases must be eliminated from our consideration, because no sodium citrate was used in the blood transfusion. We only want to mention that the cases reported by Schumacher, Denk, Haebler and Clairmont led to exitus due to hemolysis, partly under the symptoms of a severe kidney disorder (uremia). While in the case of Clairmount the blood type had been determined wrong accidentally, no determination of the blood type was made in the other cases. Suesstrunk thinks that the death of a patient who died of acture kidney insuffiency was due to the fact that the blood-transfusion acted as a new damaging moment on an existing pregnancy kidney; hemolysis can certainly be excluded, since the blood type was exactly determined. The case of Metzler is complicated by the fact that two blood transfusions were made. Receiver and donor belonged to group 4. Death occurred 20 minutes after the second transfusion, under symptoms of cardiac weakness. No autopsy was made.

Another group comprises 3 cases where defibrinated blood was infused (2 cases by Werner, 1 case by Schweitzer - reinfusion). Schweizer considers the defibrination of the blood as the cause of death. With regard to the Werner cases, it should be pointed out that death occurred during or immediately after the transfusion. Since the patients were highly enemic,

partly even moribund, it is very likely that they succumbed to the anemia. The toxicity of the defibrinated blood may also have been a cause.

Of particular interest for our problem are the 8 other cases which occurred after the transfusion (5 cases) or reinfusion (3) of sodium citrate blood. In the case of Schaeder (reinfusion) and Grossmann (reinfusioN), who received about 2 g sodium citrate with the blood, 48 - 72 hours old blood was reinfused. The cause of death must therefore obviously be found in the toxins of the old blood, not in a damage by sodium. This view is also expressed by both authors. We can not share G.H. Schneiders view who considers the Grossmann case as a damage by sodium. First of all the sodium citrate dose was very low, then it has been proven as certain for the case that 3 days old blood was among the reinfused blood which had a dark appearance, as described by the author himself. That the age of the blood is not irrelevant for a reinfusion and that toxins of the old blood which remains in the abdominal cavity in an abdominal pregnancy can be dangerous for the patient is a well-known fact (Broese).

Notso clear is the case observed by Moons. 550 cc autogenous blood and a 40% solution of sodium citrate were infused in an anemic extrauterine pregnancy. 21 hours later the patient died. Unfortunately the original report was not available to us. The review which we read does not show how much sodiumcitrate solution was added and whether the blood used was fresh or old. It is possible that a citrate overdose was the cause of death in this case; but perhaps it was again the old toxic blood.

The other five deaths aftersodium citrate infusion are described by Berand, Werner and Zimmermann. In the Berand case, no preliminary tests were made. Later it was found that the receiver and donor blood mutually hemolyzed each other. In the cases reported by Werner, no blood type determination was made. As in the two cases described above, the patients were highly anemic and died during or immediately after the transfusion. We assume that in these cases too the severe anemia was the cause of death.

But if we want to bring the transfusion in causal relation to the death, it is by no means proven that the damage was caused by sodium citrate. Hemolysis due to different blood types is more likely. Finally the two deaths reported by Zimmermann must be evaluated in the same way. Death occurred in the first patient by anemia and general infection (originating from a mastitis) and in the second patient by anemia, perhaps also hemolysis.

If we summarize the statistics of deaths after blood transfusion and reinfusion by Schneider, Breitner and our own results, we find that, except for the unclear case by Moons, the cause of death canby no means be traced back positively to damage by sodium citrate, though sodium citrate had been added to the infused blood in a number of cases. As causes of death must rather be considered: hemolysis (due to the incompatibility of blood types between donor and receiver), as well as the effect of old (reinfused) or defibrinated blood. In some case the blood transfusion (even with the addition of citrate) is not in a causal, but only in a time relation to the death which occurred due to an existing anemia.

On the basis of these findings, the reports found in the literature that citrate blood as such has led to death in transfusion should be deleted from the literature until such a case has been proven beyond any doubt.

Nevertheless there is a possibility that sodium citrate blood is not harmless for the human organism, and can lead to serious changes and disorders (Baer, G.H.Schneider, Hempel and Beck et al.). If we look at the literature, we notice the frequent indication that the transfusion of citrate blood causes more often reactions than the direct transfusion of blood with the apparatus by Oehlbecker, Percey and Beck. These data originate mostly from authors who have worked exclusively or predominantly with these apparatus. On the other hand, there is the fact that numerous series of citrate blood transfusions are reported without any minor disturbances (Schultheiss, Lewisohn, Mayo Clinic, Albert and Groulard and others). In studies by supporters of the direct blood transfusion we find reports that reactions,

frequently of a violent nature, appeared after direct blood transfusions. We are therefore of the opinion - also on the basis of our own experiences - that we must always figure with the possibility of reactions after a blood transfusion, regardless of which method is used; we are furthermore of the opinion that these disturbances, as long as they are only temporary phenomena, should not be judged too seriously, and we join Doederlein in his view that these occassional reactions must be acepted if a life can be saved.

In general it is assumed that the toxicity of the citrates is based on their tendency to bind the ionized calcium, as well as the calcium bound in the blood and in the tissues, forming insoluble salts, so that they act similar to the oxalates. In favor of this theory argue not only the chemical properties of the citrates, but also a number of other observations, like the decalcification of milk by citrates, the coagulation of the blood, made uncoagulable by citrate, by the addition of $CaCl_2$, the elimination of intoxciation symptoms caused by citrate by the addition of $CaCl_2$, and the reduction of the calcium content of the blood by the addition of citrate, observed by some authors. (Clark, Katz) As obvious as the assumption of a similar mechanism of action of the citrates and oxalates may be, there are some doubts about this concept on the basis of other observations. Gross found, for example, that citrates, unlike the oxalates, do not reduce the serum calcium; Rosenthal as well as Pickering observed an acceleration of the blood coagulation by small citrate doses without influencing the serum calcium. While the citrate is destroyed in the organism, oxalate is not burnt up in the body. Furthermore the injection of oxalate causes far reaching, partly irreparable organic changes in the organism which can be fatal even after several hours, while no such damages are known of the citrate.

Under these conditions it seemed to us necessary for our question to investigate the pharmacological action of the citrate. There are several reports available on the toxic dose of the citrate: Wohlwill indicates that 0.008 citrate per 50 g frog are toxic, 0.02 g are lethal, and mammals show

a better tolerability. Torii found for rabbits a lethal dose of 0.177 g per kg in 10% solution injected intravenously. For the dog Nuernberger calculates the lethal dose for 1 kg living weight with 0.17 to 0.24 g; according to Panisset and Verge, the toxic dose is only 0.25 g per kg, while according to Salant and Wise 0.4 to 1.5 g per kg are toxic. The latter researchers also think that the toxicity of the citrate depends on the strength of the oxidations in the organism.

Our own investigations had the following result:

I. Determination of the toxic dose for intravenously injected sodium citrate in rabbits

A 1.9 kg weight, 1 cc 4% sodium citrate solution iv. no intoxication symptoms

B 1.95 kg weight, 1 cc 8% sodium citrate solution. No intoxication symptoms

C 2.05 kg weight, 1.5 cc 8% sodium citrate solution i.v. no intoxication symptoms

D 1.9 kg weight, 2 cc 8% sodium citrate solution i.v., no intoxication symptoms

E 1.8 kg weight, 3 cc 8% sodium citrate solution i.v., convulsions, dyspnea, recovery after 5 minutes

F 2.1kg weight, 4 cc 4% sodium citrate solution, no intoxication symptoms

G 1.95 kg weight, 5 cc 4% sodium citrate solution; no intoxication symptoms

H 1.90 kg weight, 6 cc 4% sodium citrate solution, convulsions, dyspnea, recovery after 4 minutes

II. Determination of the lethal dose for intravenously injected sodium citrate in rabbits

A 1.8 kg weight, 7 cc 4% sodium citrate solution, convulsions, dyspnea, recovery in 8 minutes

B 2.05 kg weight, 8.5 cc 4% sodium citrate solution, severe convulsions, dyspena, death

C 2.2 kg weight, 10 cc 4% sodium citrate solution; death

D 1.9kg weight, 4.5 cc 8% sodium citrate solution; death

E. 1.8 kg weight, 3.0 cc 8% sodium citrate solution; death

III Determination of the toxic dose for intravenously injected sodium citrate in dogs

A 6.5 kg weight, 14 cc 8% sodium citrate solution; no intoxication symptoms

b 7.3 kg weight, 18 cc 8% sodium citrate solution; convulsions, dyspnea, recovery after 5 minutes

C 7.5 kg wt. 18 cc 8% sodium citrate solution; convulsions, dyspnea, recovery after 5 minutes

D 10.5 kg wt. 12 cc 15% sodium citrate solution; dyspenea

E 8.5 kg wt. 11 cc 15% sodium citrate solution; dyspnea, convulsions, recovery after 5 minutes

IV Determination of the lethal dose for intravenously injected sodium citrate in dogs

A 6.5 kg wt. 11.5 cm 15% sodium citrate solution; severe convulsions, dyspnea recovery after 15 minutes

B 8.5 kg wt, 16 cc 15% sodium citrate solution; death

C 10.5 kg wt, 19.5 cc 15% sodium citrate solution; death

According to these tests, the toxic dose for rabbits with i.v. injection was 0.13 g/kg, the lethal dose 0.18 g/kg, fordogs the toxic dose was 0.2 g/kg, the lethal dose 0.28 g/kg. According to the experience by Torii, the values are lower in the rapid i.v. injection of citrate solutions. As it was shown by Hatcher and Eggleston, the lethal dose does not depend only on the rapidity of the injection, but also on the concentration of the sodium citrate solution used. Torii thought, however, that the concentration obtained in the blood is determinant. Furthermore the size of the test animals is also important, as it results from a later reported test (table II). Finally, the above mentioned theory by Salant and Wise on the importance of the strength of the oxidations may also play a role.

These various factors (rapidity of the injection, concentration of the solution, rate of the oxidation of the citrate in the organism) account for

the different findings of individual authors about the toxic and lethal dose. Consequently there are no fixed doses: they depend in each case on the test conditions. Application of the conditions found in animals to man is therefore only permissible if the lowest values are used as a basis for the calculation, though it is possible that the human body can tolerate large citrate doses. From these viewpoints our tests seem particularly important, since they yielded very low values. This will be discussed more fully below.

In some of our tests we investigated the calcium content of the blood before and after the injection of a lethal dose of sodium citrate according to the method by Cramer-Tisdall.

V. Calcium content of the blood after intravenous injection of a lethal dose of sodium citrate

a) In rabbits

A.	Calcium content	5 min.	before	injection	10.3 mg%
	"	2	after	"	9.1
B	"	5	before	"	10.5
	"	2	after	"	9.0
C	"	5	before	"	10.7
	"	2	after	"	9.1

b) In dogs

A.	calcium content	5	before	"	11.2 mg%
	"	2	after	"	9.5
B	"	5	before	"	10.8
	"	2	after	"	9.4
C	"	5	before	"	11.1
	"	2	after	"	9.0

These investigations thus showed that the calcium content of the blood drops in rabbits on the average after i.v. injection of a lethal dose of sodium citrate from 10.5 to 9.1 and in dogs from 11.0 to 9.3. This finding is in agreement with the data by Clark and Rosenthal. The citrate obviously captures the free calcium-ions of the blood, but not the organically combined calcium, like the oxalate. In favor of this assumption also argues the observation that sodium citrate, unlike the oxalate, does not yield a precipitate

with diluted calcium solutions, but cancels the dissociation of the lime salts; from this behavior we must conclude that the calcium salts of the plasma are not precipitated by citrate (just as by fluoride), but remain in solution - at least partly - in non-ionized form (as complex salts). In agreement with this view is also the observation by Sluiter that citrate blood can be made to coagulate by the addition of $MgCl_2$, Oxalate blood, however, can not, the effect of Mg consisting obviously in that it is capable of releasing the complex bound calcium in the blood from its bonds and putting itself in its place.

If small doses of citrate (0.08 - 0.1 g per kg) were injected into the test animals for several days, the blood showed a considerable increase of its coagulating capacity which was so great that a test of the blood for its calcium content, for example, was impossible since the blood which was greatly diluted with distilled water still gelled. This increase of the coagulating capacity of the blood after small citrate doses,which is due according to Rosenthal on a dissolution of the blood platelets accounts for the clinical experiences of Neuhof and Hirshfeld, Renaud and Juge and others, who could arrest hemorrages in many by citrate injections.

The hypothesis by Rosenthal is shared by Higgens and Fisher who observed after intramuscular injection of sodium citrate a rapid reduction of the coagulation time in the course of 1-3 hours with a gradualreturn to the norm within 24 - 48 hours. The destruction of the blood platelets is said to release a substance which influences the coagulation process (purpura etc.) - If the blood plateles are absent the result is negative.

This hypothesis would seem apt to explain the different action of sodium citrate, which partly prevents coagulation of the blood and partly accelerates it, if certain test conditions are maintained. We therefore investigated the influence of sodium citrate on the blood platelets in a special test series. Blood smears were prepared from a number of patients (partly healthy and partly gynecologically diseased women) according to the Ponio method, and

the thrombocytes were counted (For the technique see Lowenstein (DF. Lowenstein was kind enough to do the counting). As a diluent for the blood drop issuing from the finger tip was used 1.25% and 30% sodium citrate solution in physiological salt solution and for control 14.4% magnesium sulfate solution (according to the original prescription). Since we were only interested in relative values, we only determined the number of thrombocytes found in 1000 erythrocytes.

Table I Determination of the thrombocytes according to the Fonio method by adding magnesium sulfate of sodium citrate solution of different percentage

no. of cases	magnesium sulfate 14.4%	sodium citrate 1.25%	sodium citrate 30%
1	96	99	-
2	42	-	41
3	66	41	53
4	66	70	71
5	118	99	133
6	86	79	84
7	70	57	65
8	54	71	48

From these tests it is not possible to determine a mathematical variation of the thrombocytes under the action of sodium citrate solution. The fluctuations, which show both an increase and a decrease, are probably within the error margin.

In order to be sure that these findings are not based on a faulty technique and particularly in order to determine the processes taking place in the blood vessels themselves after intravenous injection of sodium citrate, we also made animal tests. Two rabbits were given intravenous sodium citrate injections; before and after the injection magnesium sulfate smears were prepared from the shaved ear skin to count the thrombocytes.

The animsl tests do not show either a regular reduction of the thrombocytes on the contrary; one could be inclined to assume an increase. However we think that this increase is only an apparent one, since in our experience the admixture of sodium citrate to the blood preserves the thrombocytes better and arrests their decomposition.

Table II

no. of thrombocytes before injection	injection of	no. of thrombocytes after injection	behavior of animals
	Animal no. I, 3750 g		
56	16 cc of a 5% solution (0.8 g)	70	exitus under dyspnea and convulsions after a few minutes
	Animal no. II, 3200 g		
45	2 cc of a 30% solution (0.6 g)	53	no reaction
5 min. later	same dose (0.6 g)	47	rest
5 min. later	6 cc of a 5% solution (0.3 g)	56	convulsions, dyspnea recovery
10 min. later	same dose (0.3 g)	61	convulsions severe dyspnea recovery

In a comparison of our smears obtained from man, which had been prepared partly by the addition of magnesium sulfate and partly by the addition of sodium citrate, we observed repeatedly that the thrombocytes can be much better recognized in the citrate preparation and were partly better preserved than in the magnesium sulfate preparation.

According to these tests, the theory by Rosenthal seems to us untenable. An explanation for the increased blood coagulation by repeated small doses of sodium citrate can not be given at the present time. In this connection it should be mentioned that Renaud and Juge think that the hemostatic effect of a sodium citrate injection is due to a variation of the vasomotors and to the enhancement of the formation of thrombi in the hemorrhaging tissue.

In our tests described above a pure sodium citrate solution was injected into the test animals. But the conditions in a blood transfusion are different insofar as the sodium citrate used here is not introduced in pure form, but mixed with the donor's blood. The question arises thus whether the entire amount of citrate used acts on the organism. We made therefore the following tests: We took from an animal (rabbit, dog) 10-20 cc blood from the jugular vein into which a lethal dose of citrate for this animal had been introduced. This blood, which had become uncoagulable by the citrate, was

injected intravenously after 2 minutes into the animal. Toxic symptoms appeared (convulsions, dyspnea); but after these symptoms had disappeared within ½ minute, the animals showed again a normal behavior. From these findings it can be concluded that not the entire amount of citrate added to the donor blood had acted harmfully on the receiver organism; a part is adsorbed by the donor blood and is thus made ineffective.

With regard to the effects of the citrate on respiration, blood pressure, peristaltic, diuresis etc. we refer to the pertinent literature. But it should be mentioned that only pure trisodium citrate may be used blood transfusions. Thus it was found after the addition of equal amounts of mono- and trisodium citrate (1 cc each of a 20% solution to 5 cc blood), that blood mixed with trisodium citrate had not yet coagulated after 6 hours and shows a bright red color, while, when the same amount of sodium citrate is used, the blood coagulates in small clots and assumes a chocolate-brown color (effect of acid, formation of hemin). With this experience we verify the data by v. Wolff.

We now have to discuss the question whether the addition of sodium-citrate can cause a change in the immunity reactions of the blood as well as damages of the blood elements.

An important factor in the production of shock-type symptoms is the reduction or disappearance of the complement function. We know that sodium citrate influences this function in the sense that it prevents the bonding of complement on the blood and hemolytic immune bodies and thus the appearance of hemolysis (Gengou). Lewisohn also found that an inhibition of the phagacytosis does not take place when sodium citrate is added to the blood. Jeaubrau indicates that antibodies and endocrine substances are not destroyed. According to investigations by Cook, the i.v. injection of sodium citrate in rabbits leads to a faster and more intensive formation of precipitin and to a considerable increase of the formation of antibodies, compared to the control animals, while the intravenous injection of $CaCl_2$ greatly inhibits these

processes. Citrate has no inhibiting effect on the bactericidal action of the blood either, according to the investigations by Walsh and Harmsworth.

In the literature we find frequently the information that the blood elements are damaged by a citrate blood infusion: The resistance of the blood corpuscles is reduced, red blood corpusclules and the blood platelets are damaged (Hemple Beck). These contentions can not remain uncontradicted. Jeaubrau reports that the blood freezing point, the viscosity coefficient, and the respiratory capacity of the red blood corpuscles remain unchanged. Clarimont and M.A. Mueller indicated recently that sodium citrate does not change all blood elements if an isotonic (4%) solution is infused. Lewisohn rejects a destruction of the red corpuscles; H. Opitz found in his investigations that the resistance of the erythrocytes is by no means reduced; Torii found in this test that the number of the red corpuscules is not substantially changed, even after toxic doses.

These tests and experiences thus do not provide any reason for the assumption that the immunizatory processes in the organism are inhibited by sodium citrate; the present observations rather suggest a favorable influence. Furthermore a harmful effect on the blood elements is by no means proven.

Normet thinks that the toxic effect of large citrate doses is due to the excitation of the vegetative nervous system. This exciting action is not caused by the citrate -ion, but by the sodium-ion. The electrically negative radical acid is adsorbed by the blood colloids, the sodium-ion is released, which in turn displaces other positive ions of the tissue. Our objection to this theory is as follows: it is not known that the injection of sodium ions in concentrations, as they are used in the injection of toxic doses of sodium citrate, leads to states of excitation of the vegetative nervous system. Besides, the colloids of the blood and of the tissues are charged negatively intra vitam, so that they would adsorb the sodium primarily if the adsorption took place on the basis of electrical processes; but if the

adsorption does not take place according to the rules of the polar adsorption, this can only be an exchange adsorption on the basis of chemical affinities where the citrate-ion displaces another negative ion, which in turn is bonded by the sodium-ion of the citrate.

We have discussed in detail the possibility of an injury of the organism in the injection of sodium citrate, because only by clarifying this question can a base for the same therapeutic application of sodium citrate in blood transfusions be provided. Our animal tests and literature studies have shown again that sodium citrate is only dangerous in certain doses and that it can even be lethal under certain circumstances, but that a citrate blood transfusion is safe if toxic doses are avoided, and that the transfused blood as well as the blood of the receiver do not suffer any harmful changes. In the practical realization of a citrate blood transfusion it is therefore important to use safe citrate doses, that is, to remain below the toxic dose. (That an overdose can produce life-threatening symptoms is proven by the case of Shinner, where a 30% sodium citrate solution instead of a 3% sodium citrate is necessary to prevent coagulation of the donor blood. This therapeutic range of sodium citrate must be known and maintained; then there is no cogent reason to forego its use in blood transfusions (Wuerberger).

Nuernberger found in thorough investigations that 0.2 g sodium citrate is already sufficient to prdect 100 cc blood from coagulating. This amount represents a minimum; 0.175 g no longer prevent coagulation. In practical use it is therefore advisable to selectthe sodium citrate concentration slightly higher, since too much blood may be easily used in the preparation of the donor blood solution. The toxic dose of sodium citrate according to our test results, which must be considered very low compared to the results of other authors, is 0.13 g/kg for the rabbit and 0.2 g for the dog. Based on these doses, the toxic dose for a person weighing 50 kg would be 6.5 - 10 g sodium citrate. Nuerberger, who based his calculations on a

test on a dog, where the exact amount of sodium citrate used was not known, calculated as a toxic dose for a person of 65 kg 11-15 g sodium citrate. Our calculation thus represents a very low dose. This is also proven by the tests by Renaud and Juge, who injected sodium citrate intravenously in man to stop various hemmorhages. They used 10-25 cc of a 30% solution, that is, 3-7.5 g. Pasti reports about similar tests. He increased his dose up to 9 g, which he injected, however, intramuscularly.

How high are the amounts of sodium citrate used in actual practice in blood transfusions? According to Bumm 500 cc blood are mixed with 200 - 300 cc 1% sodium-citrate solution in 0.9%table salt solution, that is, 2-3 g sodium citrate are added to 500 cc blood. In the transfusion of 1000 cc blood (= 1400- 1600 cc liquid) the receiver receives 4-6 g sodium citrate. Only with this rather large amount of transfusion is the toxic minimum dose calculated by us attained in the maximum case.

Doederlein uses a mixture of 300 cc blood + 200 cc 1% sodium citrate-solution, that is, 2 g per 300 cc blood, 6.7 g per 1000 cc blood. Other authors used smaller doses.

At the Stoeckel Clinic the following formula is used: 250 cc normosal-solution are mixed with 25 cc of a 5% sodium citrate solution, and 250 cc donor blood are added. The receiver receives therefore with 1000 cc blood (2000 cc liquid) 5 g sodium citrate, a dose which is still under the toxic minimum dose and therefore certainly safe.

In most cases it suffices to transfuse 1 liter donor blood to successfully fight a life-threatening anemia. Most authors recommend 500 - 800 cc (Stickel et al.) Together with the blood, a considerable amount of diluting liquid (table salt solution or better normosal solution) is incorporated in the receiver; it naturally increases the therapeutic effect to fill up the blood vessel system again. In case it is desired to transfuse less diluting liquid but more blood, that is, to give the receiver more than 5-6 sodium citrate, there is no danger at first, because our tests have shown

that a part of the sodium citrate which is added to the blood to make it uncoagulable is made harmless for the receiver. The "toxic minimum dose" which we have calculated for man applies only to the case where a pure solution of sodium citrate, as in the animal test, is injected in man. But since the concentration used is very important for the toxicity of the citrate, as indicated above, it is advisable to use low citrate concentration in citrate blood transfusion (possibly by adding normosal solution to the donor blood). If these precautions are maintained, there is no danger for the receiver on the basis of the animal experiments and observation in man, as well as on the basis of theoretical considerations.

The last question to be clarified is whether the tolerability for sodium citrate is changed after great blood losses (G.H.Schneider). Experimental studies on this subject are available by Joannides and Cameron. These authors found that hemorrhages do not increase the toxicity of the sodium citrate; but they observed that a 0.2% citrate solution already suffices to increase the blood pressure and the vital activity. On the basis of these results the two authors recommend citrate blood transfusion for man too.

Summarizing we arrive at the following conclusions:

1. Deaths after sodium citrate blood transfusions, which were caused by the addition of sodium citrate to the donor blood, have not been observed so far, except in the case of gross errors (accidental overdosage). The deaths reported in the literature are due to incompatibilities of the receiver- and donor blood, as well as to the toxic effect of the transfused defibrinated or old blood.

2. The toxic minimum dose of sodium citrate is calculated according to our tests for the rabbit with 0.13 g/kg weight, and for the dog with 0.2 g/kg. The lethal dose is about 50% higher. In the toxic effect, the concentration of sodium citrate in the blood plays an important role. The slower the injection, the lower the concentration of sodium citrate in the injection liquid,

the lower is the toxicity.

3. In the transfusion of sodium citrate blood, a part of the sodium citrate is made ineffective by the donor blood. In our own tests we found that the otherwise lethal sodium citrate dose no longer causes death under these conditions.

4. Small doses of sodium citrate, injected repeatedly for several days, increase the coagulating capacity of the blood. Destruction of thrombocytes does not seem to play a role here.

5. After intravenous injections of a lethal dose of sodium citrate, the calcium content of the blood drops in the rabbit from 10.5 to 9.1 mg%, in the dog from 11.0 to 9.3 mg%. The citrate obviously confiscates the free calcium-ions of the blood, but not the organically combined calcium.

6. Calculated for a person weighing 50 kg, the toxic minium dose for man results with 6.5 - 10 g sodium citrate. This dose is tolerated without any harm by man, as clinical experiences show.

7. In the practical realization of blood transfusions with sodium citrate 5 g sodium citrate are used for 1 liter blood, 10 g for 2 liter (method: Stoeckel Clinic).

8. Variations of the immunizatory processes in the blood, as well as of the blood elements in sodium citrate solutions are not likely, as long as the in0 dicated doses are not exceeded.

9. The transfusion of sodium citrate blood is safe, provided overdosing is avoided.

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(Translated by Carl Demrick Associates, Inc/IE/t)

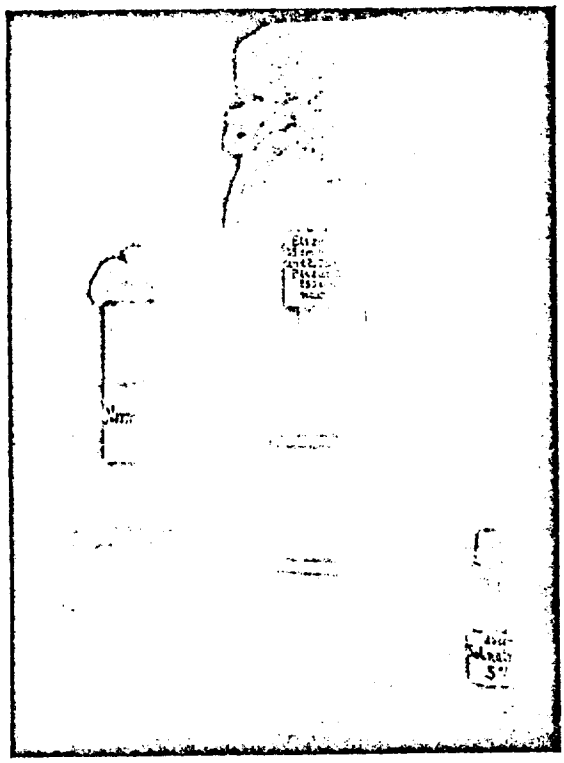

Figure 1

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XXXIII.

Aus der Universitätsfrauenklinik (Direktor: Geh.-Rat Stoeckel)
und dem Pharmakologischen Institut Berlin
(Stellvertretender Direktor: Prof. Joachimoglu).

Zur Frage der Ungefährlichkeit von Zitratbluttransfusionen.

Experimentelle Untersuchungen über die toxische und letale Dosis des Natriumzitrats sowie über seinen Einfluß auf den Blutkalkspiegel und die Thrombozyten.

Von F. v. Mikulicz-Radecki und E. Keeser.

Mit 1 Abbildung.

Die Transfusion von Natriumzitratblut ist ohne eine größere Apparatur möglich. Wenn trotzdem die Natriumzitratmethode in Deutschland — ganz im Gegensatz zu Amerika und Frankreich — sich keiner großen Beliebtheit erfreut, so ist dies auf die weit verbreitete Ansicht zurückzuführen, Natriumzitratblut sei nicht mehr vollwertig bzw. das mit dem Blut einverleibte Na-

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triumzitrat schädige den Empfänger. Eine nähere Begründung für diese Ansicht bleiben aber die meisten Autoren schuldig (z. B. G. H. Schneider, W. Baer, P. Schumacher u. a.).

Die Notwendigkeit, eine Bluttransfusion schnell vorzunehmen, ist in der inneren Medizin — und auch in der Chirurgie — viel seltener gegeben als in der Geburtshilfe. Der Geburtshelfer (dazu rechnen wir auch den Gynäkologen, der eine geplatzte Extrauteringravidität operiert) hat bei strenger Indikation keine Zeit zu verlieren und benötigt eine Methode, die absolut sicher und rasch eine Transfusion ermöglicht. Sie muß von jedem chirurgisch geschulten Arzt ausgeführt werden können und keine spezielle Übung erfordern; sie muß weiter ohne Koppelung des Spenders an den Empfänger ausführbar sein, und sie muß schließlich mit einer gewöhnlichen Kochsalzinfusion kombiniert werden können. Von der Erfüllung dieser Forderungen hängt das rasche Gelingen einer Bluttransfusion in der Geburtshilfe ab.

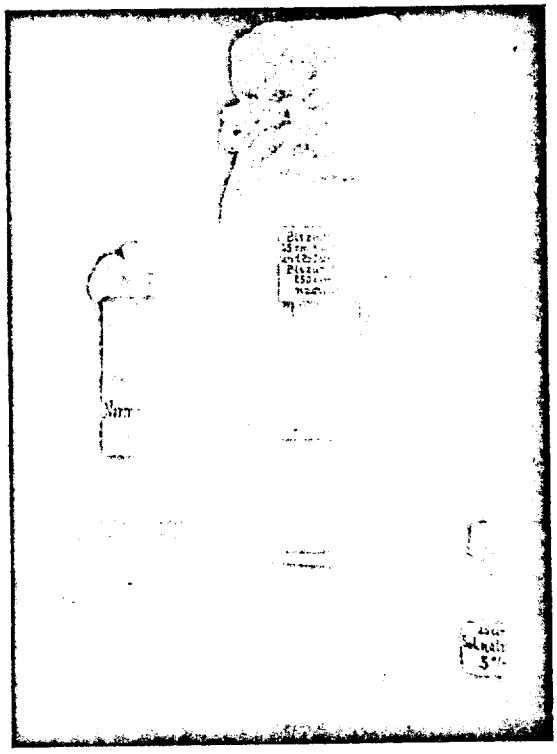
Bei strenger Indikation ist eine Bluttransfusion auch bei einem größeren geburtshilflich-gynäkologischen Material nur in seltenen Fällen nötig. Es ist daher unmöglich, daß z. B. an einer Klinik eine größere Anzahl von Ärzten die Technik eines komplizierten Transfusionsapparates (Oehlecker, Percey, Beck u. a.) perfekt beherrscht, so daß stets ein Geübter zur Verfügung steht. Jeder Transfusionsapparat hat aber seine Tücken, wie aus zahlreichen Arbeiten hervorgeht. Weiter ergibt sich bei strenger Auswahl der Fälle, daß sehr viel häufiger eine intravenöse Kochsalzinfusion zum Blutersatz ausgeführt wird; nicht so selten ergibt sich erst während ihrer Ausführung die Notwendigkeit einer Bluttransfusion aus dem weiteren klinischen Verlauf. Schließlich hat die Verwendung aller Transfusionsapparate, bei denen Spender und Empfänger eng verbunden sein müssen, den großen Nachteil, daß Operationen, die gleichzeitig ausgeführt werden müssen, stark behindert sind. Muß gar die Patientin während der Bluttransfusion aus dem Bett auf den Operationstisch gelegt oder auf dem Operationstisch umgelegt werden (z. B. Übergang vom vaginalen Operieren zum abdominalen), so muß eine direkte Bluttransfusion unterbrochen werden; die gleichzeitige Umlagerung des Spenders bereitet Schwierigkeiten, sie kann gelegentlich wegen der räumlichen Verhältnisse unmöglich sein. Diese, hier nur kurz skizzierten Nachteile fallen bei der Zitratbluttransfusion fort. Diese beginnt mit einer intravenösen Kochsalzinfusion; in einem Nebenraum wird die Transfusionsflüssigkeit gewonnen und vorbereitet, wobei dem Spender der deprimierende Eindruck des Kampfes um ein Menschenleben erspart bleibt. Durch Einfüllung des Zitratblutes in die Kochsalzbürette wird die Kochsalzinfusion in eine Bluttransfusion verwandelt, deren Größe nach Bedarf variiert werden kann. Schließlich kann trotz Kochsalzinfusion jede Umlagerung der Patientin leicht vorgenommen werden.

Bezeichnend für die praktischen Bedürfnisse in der Geburtshilfe ist die

Tatsache, daß zwei führende Geburtshelfer, Bumm und Döderlein, warm für die Zitratblutmethode eingetreten sind. Auch die Stoeckelsche Klinik verwendet sie allein seit Jahren, nachdem mannigfache Versuche mit direkten Transfusionsapparaten nicht befriedigt hatten.

Abb. 1 gibt die Apparatur wieder, die an der Stoeckelschen Klinik bei der Vornahme einer Zitratbluttransfusion gebraucht wird. An einem 1000-ccm-Meßzylinder sind mit dünnen Leukoplaststreifen zwei Marken angebracht. Wie aus der Aufschrift hervor-

Abb. 1.



Apparatur zur Vornahme einer Zitratbluttransfusion an der Stoeckelschen Klinik.

geht, soll der Meßzylinder bis zur ersten Marke mit 25 ccm 5%igem Natrium citricum und 250 ccm Normosallösung aufgefüllt werden. Da in dem Natrium-citricum-Fläschchen bereits 25 ccm abgemessen sind, wird zunächst diese Flüssigkeitsmenge in den Meßzylinder gegossen, sodann Normosallösung bis zur ersten Marke nachgefüllt; damit ist genau die Menge von 250 ccm erreicht.

Nach nunmehriger Punktion der Vena cubitalis beim Spender wird durch die Punktionskanüle das Blut in den Meßzylinder übergeleitet, bis die Flüssigkeitsmenge die zweite Marke erreicht. Damit sind genau 250 ccm Spenderblut gesammelt und eine 0,25%ige Natrium-citricum-Blutlösung hergestellt. Sollte versehentlich etwas mehr Blut übergeleitet worden sein, so schadet das nichts; die Natrium-citricum-Konzentration ist so hoch bemessen, daß bei geringem Überschreiten des Mischungsverhältnisses eine Gerinnung nicht eintritt. Der Überschuß an Blut kann an der Graduierung des Meßzylinders leicht

abgelesen werden. Das Zitratblutgemisch wird nicht umgerührt, sondern nur im Meßzylinder leicht geschüttelt.

Wir glauben, daß mit der Zeit auch in Deutschland die Zahl derjenigen, die die Zitratmethode bei Bluttransfusion verwenden, wachsen wird, genau wie in Amerika, wo z. B. die Mayosche Klinik auf Tausende von Zitratbluttransfusionen zurückblicken kann. Voraussetzung dafür ist allerdings der Nachweis, daß das Zitratblut keine nennenswerten Störungen hervorruft. Um diesen Nachweis zu erbringen, muß 1. die Frage beantwortet werden, ob Todesfälle nach Bluttransfusionen bzw. Reinfusionen bekannt sind, die lediglich der Beimengung von Natriumzitrat zum transfundierten Blut zuzuschreiben sind, 2. ist im Tierversuch zu prüfen, wie sich das Versuchsobjekt nach Einverleibung von Natriumzitrat verhält.

Wir haben versucht, aus der Literatur möglichst genau die Todesfälle nach Bluttransfusion zusammenzustellen. Hierüber liegen bereits zwei Sammelstatistiken vor: eine Arbeit von P. Schneider aus dem Jahre 1925, der über 15 Todesfälle aus den letzten 4 Jahren berichtet, und eine Zusammenstellung von Transfusionstodesfällen in der Breitnerschen Monographie. Schneider konnte den Nachweis erbringen, daß alle von ihm zusammengestellten Todesfälle auf eine Unstimmigkeit der Blutgruppe von Spender und Empfänger zurückzuführen sind. Niemals wurden die Spender so ausgewählt, daß sie derselben Blutgruppe wie die Empfänger angehörten. Entweder wurde überhaupt nicht auf Agglutination geprüft bzw. es wurde bei der Prüfung ein Fehler gemacht; oder es wurde nur eine Prüfung auf gegenseitige Agglutination angestellt (große gegenseitige Agglutinationsprüfung oder Nürnbergersche Probe), was nach den heutigen Anschauungen nicht sicher Isoagglutinine ausschließt. Die Todesursache bestand in den von Schneider nachgeprüften Fällen in Hämolyse, bis auf den von Carrington, Lunsford und Lec beschriebenen Fall, bei dem der Tod unter dem Bilde eines anaphylaktischen Schocks eintrat. Bei den von Breitner gesammelten Fällen (18 Autoren) konnte gleichfalls gezeigt werden, daß der Tod durch Unstimmigkeit der Blutarten zustande kam. Diesen Todesfällen der Statistiken P. Schneider und Breitner können wir 18 weitere in der Literatur beschriebene hinzufügen, darunter auch einige Todesfälle nach Reinfusion.

Bei 7 Fällen wurde eine direkte Bluttransfusion mit dem Oehleckerschen oder Perceyschen Apparat ausgeführt (2 Fälle von Schumacher, je 1 Fall von Denk, Haebler, Clairmont, Metzler und Süßtrunk); diese scheiden für unsere Fragestellung von vornherein aus, da bei der Bluttransfusion kein Natrium citricum verwandt wurde. Es sei nur erwähnt, daß die von Schumacher, Denk, Haebler und Clairmont mitgeteilten Fälle infolge Hämolyse, zum Teil unter Erscheinungen schwerer Nierenschädigung (Urämie) ad exitum kamen. Während bei dem Fall von Clairmont aus Versehen die Blutgruppe falsch bestimmt

worden war, war bei den anderen Fällen eine Blutgruppenbestimmung unterblieben. Süßtrunk führt den Tod seiner Patientin, die an akuter Niereninsuffizienz starb, darauf zurück, daß die Bluttransfusion als neues schädigendes Moment auf eine bereits bestehende Schwangerschaftsniere einwirkte; eine Hämolyse ist sicher auszuschließen, die Blutgruppe wurde genau bestimmt. Der Fall von Metzler ist dadurch kompliziert, daß zwei Bluttransfusionen ausgeführt wurden. Empfänger und Spender gehörten der Gruppe 4 an. Der Tod trat 20 Minuten nach der zweiten Transfusion unter Erscheinungen von Herzschwäche ein. Eine Sektion unterblieb.

In eine weitere Gruppe fassen wir 3 Fälle, denen defibriniertes Blut infundiert wurde (2 Fälle von Werner, 1 Fall von Schweitzer — Reinfusion). Schweitzer sieht als Todesursache das Defibrinieren des Blutes an; für die Wernerschen Fälle ist zu bemerken, daß der Tod während oder sofort nach der Transfusion eintrat. Da es sich um stark ausgeblutete, zum Teil moribunde Patientinnen handelte, erscheint es wahrscheinlich, daß sie der Anämie erlegen sind. Außerdem kommt als Todesursache die Giftigkeit des defibrinierten Blutes in Betracht.

Für unsere Fragestellung sind besonders die 8 übrigen Todesfälle von Interesse, da sie nach der Transfusion (5 Fälle) bzw. Reinfusion (3 Fälle) von Natriumzitratblut eintraten. Bei Fall Schäfer (Reinfusion) und Fall Großmann (Reinfusion), die mit dem Blut etwa 2 g Natriumzitrat erhielten, wurde 48—72 Stunden altes Blut reinfundiert. Die Todesursache ist daher offenbar in den Toxinen des alten Blutes zu suchen, nicht in einer Schädigung durch das Natrium citricum. Diese Ansicht wird auch von den beiden Autoren ausgesprochen. Den Ausführungen G. H. Schneiders, der den Fall Großmann als eine Schädigung durch Natrium citricum ansieht, können wir uns nicht anschließen. Einmal war die Natrium-citricum-Dosis recht gering; weiter ist für den Fall sicher erwiesen, daß unter dem reinfundierten Blut sich 3 Tage altes Blut befand, das, wie der Autor selbst beschreibt, dunkel ausgesehen hat. Daß das Alter des Blutes für eine Reinfusion nicht gleichgültig ist, ja, daß Toxine des alten Blutes, das bei einer geplatzten Bauchhöhlenschwangerschaft in der Bauchhöhle zurückbleibt, für die Trägerin gefährlich werden können, ist zur Genüge bekannt (Bröse).

Nicht so eindeutig ist der von Moons beobachtete Fall: Bei einer ausgebluteten Extrauteringravidität wurden 550 ccm Eigenblut und eine 40%ige Lösung von Natrium citricum infundiert. 21 Stunden danach starb die Patientin. Leider war uns die Originalarbeit nicht zugänglich. Aus dem uns vorliegenden Referat ist nicht zu ersehen, wieviel Natrium-citricum-Lösung zugesetzt wurde, ferner ob das verwandte Blut frisch oder alt war. Man könnte bei diesem für uns ungeklärten Fall an eine Zitratüberdosierung als Todesursache denken; vielleicht handelt es sich aber um bereits altes, toxisch wirkendes Blut.

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Die übrigen 5 Todesfälle nach Natriumzitratinfusion sind von Bérand, Werner und Zimmermann beschrieben. Bei dem Fall von Bérand sind keine Vorproben ausgeführt worden. Nachträglich wurde festgestellt, daß Empfänger- und Spenderblut sich gegenseitig hämolysierten. Bei den von Werner mitgeteilten Todesfällen unterblieb gleichfalls eine Blutgruppenbestimmung. Es handelt sich, wie bei den beiden oben erwähnten Todesfällen von Werner, gleichfalls um schwer anämische Patienten, die während oder sofort nach der Transfusion ad exitum kamen. Wir möchten annehmen, daß auch bei diesen Fällen die schwere Anämie die Todesursache war. Will man aber die Transfusion mit dem Tod in ursächlichen Zusammenhang bringen, so ist eine Schädigung durch Natriumzitrat keineswegs erwiesen; viel wahrscheinlicher ist eine Hämolyse infolge Blutgruppenungleichheit. In gleicher Weise sind schließlich die von Zimmermann beschriebenen zwei Todesfälle zu bewerten. Es unterblieben Blutgruppenbestimmungen. Der Tod trat bei der ersten Patientin infolge Anämie und einer allgemeinen Infektion (ausgehend von einer Mastitis), bei der zweiten infolge Anämie, vielleicht auch Hämolyse ein.

Fassen wir die Ergebnisse der Statistiken von Todesfällen nach Bluttransfusion und Reinfusion von Schneider, Breitner und uns zusammen, so ist festzustellen, daß bis auf den unklaren Fall von Moons in keinem Fall die Todesursache eindeutig auf eine Schädigung durch Natriumzitrat zurückzuführen ist, obwohl in einer großen Zahl dieser Fälle dem infundierten Blut Natriumzitrat zugefügt war. Als Todesursachen sind vielmehr anzusehen: Hämolyse (infolge von Blutgruppenunstimmigkeit zwischen Spender und Empfänger) sowie die toxische Wirkung von altem (reinfundiertem) oder defibriniertem Blut. In einigen Fällen steht die Bluttransfusion (auch bei Zitratzusatz) nicht in kausalem, sondern nur in zeitlichem Zusammenhang mit dem Tode, der infolge der bestehenden Anämie eintrat.

Auf Grund dieser Feststellungen dürfte die Angabe, daß Zitratblut als solches bei Transfusionen den Tod herbeigeführt hat, aus der Literatur zu streichen sein, bis ein wirklich einwandfreier Fall mitgeteilt ist.

Trotzdem bleibt die Möglichkeit bestehen, daß Natriumzitratblut für den menschlichen Organismus nicht harmlos ist, sondern zu bedenklichen Veränderungen und Störungen führen kann (Baer, G. H. Schneider, Hempel, Beck u. a.). Überblicken wir die Literatur, so fällt die häufige Angabe auf, daß die Transfusion von Zitratblut viel öfter Reaktionen hervorruft, als die direkte Blutüberleitung mit den Apparaten von Oehlecker, Percey und Beck. Diese Angaben stammen meist von Autoren, die nur oder vorwiegend mit diesen Apparaten gearbeitet haben. Demgegenüber steht die Tatsache, daß zahlreiche Serien von Zitratbluttransfusionen ohne nennenswerte Störungen mitgeteilt sind (Schultheiß, Lewisohn, Mayosche Klinik, Albert und Groulard u. a.). Ander-

seits wird auch in den Arbeiten der Anhänger der direkten Bluttransfusion angegeben, daß gelegentlich Reaktionen, bisweilen heftiger Natur, nach der direkten Bluttransfusion aufgetreten sind. Wir sind daher — auch auf Grund unserer eigenen Erfahrungen — der Ansicht, daß man stets mit der Möglichkeit von Reaktionen nach einer Bluttransfusion, ganz gleichgültig, welche Methode man anwendet, zu rechnen hat; wir stehen weiter auf dem Standpunkt, daß man diese Störungen, solange es sich nur um vorübergehende Erscheinungen handelt, nicht zu schwer bewerten darf, und schließen uns der Ansicht Döderleins an, daß man diese gelegentlichen Reaktionen in Kauf nehmen kann, wenn damit die Rettung des Lebens erzielt wird.

Im allgemeinen wird angenommen, daß die Giftigkeit der Zitrate auf ihrer Neigung beruht, das ionisierte sowohl wie das im Blut und in den Geweben gebundene Kalzium unter Bildung von unlöslichen Salzen zu binden, also analog den Oxalaten zu wirken. Für diese Annahme sprechen nicht nur die chemischen Eigenschaften der Zitrate, sondern auch eine Reihe von anderen Beobachtungen, wie die Entkalkung der Milch durch Zitrat, die Gerinnung des durch Zitrat ungerinnbar gemachten Blutes bei Zusatz von $CaCl_2$, die Aufhebung der durch Zitrat hervorgerufenen Vergiftungserscheinungen durch $CaCl_2$-Zufuhr sowie eine von manchen Autoren (Clark, Katz) beobachtete Erniedrigung des Kalziumgehaltes des Blutes durch Zitrat. So naheliegend daher die Annahme eines analogen Wirkungsmechanismus der Zitrate und Oxalate ist, so erheben sich doch auf Grund anderer Beobachtungen auch Bedenken gegen diese Auffassung: Groß fand z. B., daß Zitrate im Gegensatz zu den Oxalaten das Blutkalzium nicht vermindern; Rosenthal beobachtete ebenso wie Pickering eine Beschleunigung der Blutgerinnung nach kleinen Zitratdosen ohne Beeinflussung des Blutkalziums. Während das Zitrat im Organismus zerstört wird, verbrennt Oxalat im Körper nicht. Ferner verursacht die Injektion von Oxalat tiefgreifende, zum Teil irreparable organische Veränderungen im Organismus, die noch nach vielen Stunden zum Tode führen können, während vom Zitrat derartige Schädigungen nicht bekannt sind.

Unter diesen Umständen erschien es uns für die Beantwortung unserer Fragestellung notwendig, die pharmakologische Wirkung des Zitrats genauer zu untersuchen. Über die toxische Dosis des Zitrats liegen mehrere Mitteilungen vor: Wohlwill gibt an, daß 0,008 g Zitrat pro 50 g Frosch toxisch, 0,02 g letal wirken und Säugetiere eine bessere Verträglichkeit zeigen. Torii fand für Kaninchen als letale Dosis 0,177 g pro kg, in 10%iger Lösung intravenös injiziert. Für den Hund errechnet Nürnberger die tödliche Dosis für 1 kg Lebendgewicht auf 0,17 bis 0,24 g; nach Panisset und Verge liegt die toxische Dosis erst bei 0,25 g pro kg, während nach Salant und Wise erst 0,4 bis 1,5 g pro kg toxisch sind. Die letzteren Untersucher glauben, daß die Giftigkeit des Zitrats von der Stärke der Oxydationen im Organismus abhängt.

Unsere eigenen Untersuchungen ergaben folgendes:

I. Bestimmung der Dosis toxica für intravenös injiziertes Natriumzitrat bei Kaninchen.

A. 1,9 kg schwer: 1 ccm 4%ige Natriumzitratlösung intravenös.
Keine Vergiftungserscheinungen.

B. 1,95 kg schwer: 1 ccm 8%ige Natriumzitratlösung.
Keine Vergiftungserscheinungen.

C. 2,05 kg schwer: 1,5 ccm 8%ige Natriumzitratlösung intravenös.
Keine Vergiftungserscheinungen.

D. 1,9 kg schwer: 2 ccm 8%ige Natriumzitratlösung intravenös.
Keine Vergiftungserscheinungen.

E. 1,8 kg schwer: 3 ccm 8%ige Natriumzitratlösung intravenös.
Krämpfe, Dyspnöe. Nach 5 Minuten Erholung.

F. 2,1 kg schwer: 4 ccm 4%ige Natriumzitratlösung.
Keine Vergiftungserscheinungen.

G. 1,95 kg schwer: 5 ccm 4%ige Natriumzitratlösung.
Keine Vergiftungserscheinungen.

H. 1,90 kg schwer: 6 ccm 4%ige Natriumzitratlösung.
Krämpfe, Dyspnöe. Nach 4 Minuten Erholung.

II. Bestimmung der Dosis letalis für intravenös injiziertes Natriumzitrat bei Kaninchen.

A. 1,8 kg schwer: 7 ccm 4%ige Natriumzitratlösung.
Krämpfe, Dyspnöe. Erholung in 8 Minuten.

B. 2,05 kg schwer: 8,5 ccm 4%ige Natriumzitratlösung.
Schwere Krämpfe, Dyspnöe, Tod.

C. 2,2 kg schwer: 10 ccm 4%ige Natriumzitratlösung.
Tod.

D. 1,9 kg schwer: 4,5 ccm 8%ige Natriumzitratlösung.
Tod.

E. 1,8 kg schwer: 4,0 ccm 8%ige Natriumzitratlösung.
Tod.

III. Bestimmung der Dosis toxica für intravenös injiziertes Natriumzitrat bei Hunden.

A. 6,5 kg schwer: 14 ccm 8%ige Natriumzitratlösung.
Keine Vergiftungserscheinungen.

B. 7,3 kg schwer: 18 ccm 8%ige Natriumzitratlösung.
Krämpfe, Dyspnöe. Nach 5 Minuten Erholung.

C. 7,5 kg schwer: 18 ccm 8%ige Natriumzitratlösung.
Krämpfe, Dyspnöe. Nach 5 Minuten Erholung.

D. 10,5 kg schwer: 12 ccm 15%ige Natriumzitratlösung.
Dyspnöe.
E. 8,3 kg schwer: 11 ccm 15%ige Natriumzitratlösung.
Dyspnöe, Krämpfe. Nach 5 Minuten Erholung.

IV. Bestimmung der Dosis letalis für intravenös injiziertes Natriumzitrat bei Hunden.

A. 6,5 kg schwer: 11,5 ccm 15%iges Natriumzitrat.
Schwere Krämpfe, Dyspnöe. Erholung in 15 Minuten.
B. 8,5 kg schwer: 16 ccm 15%ige Natriumzitratlösung.
Tod.
C. 10,5 kg schwer: 19,5 ccm 15%ige Natriumzitratlösung.
Tod.

Nach diesen Versuchen fanden wir bei langsamer intravenöser Injektion von Trinatriumzitrat für Kaninchen die Dosis toxica bei 0,13 g pro kg, die Dosis letalis bei 0,18 g pro kg, für Hunde die Dosis toxica bei 0,2 g pro kg, die Dosis letalis bei 0,28 g pro kg. Entsprechend der Erfahrung von Torii liegen bei der schnellen intravenösen Injektion von Zitratlösungen die Werte niedriger. Wie Hatcher und Eggleston zeigten, ist die Dosis letalis aber nicht nur von der Schnelligkeit der Injektion, sondern auch von der Konzentration der verwendeten Natriumzitratlösung abhängig. Torii glaubt allerdings, daß die im Blut erzielte Konzentration entscheidend ist. Weiter ist auch die Größe der Versuchstiere von Bedeutung, wie aus einem später mitgeteilten Versuch (Tabelle II) hervorgeht. Schließlich mag auch die oben erwähnte Theorie von Salant und Wise über die Bedeutung der Stärke der Oxydationen eine Rolle spielen.

Diese mannigfachen Faktoren (Schnelligkeit der Ausführung der Injektion, Konzentration der Lösung, Schnelligkeit der Oxydation des Zitrates im Organismus) erklären die voneinander abweichenden Befunde einzelner Autoren über die Dosis toxica und letalis. Demnach gibt es keine feststehenden Dosen; sie richten sich jeweils nach den Versuchsbedingungen. Eine Übertragung der bei Tieren gefundenen Verhältnisse auf den Menschen ist daher nur dann statthaft, wenn der Berechnung die niedrigsten Werte zugrunde gelegt werden, wenn auch möglich ist, daß der menschliche Körper größere Zitratdosen zu vertragen imstande ist. Unter diesen Gesichtspunkten erscheinen unsere Versuche besonders wichtig, da sie sehr niedrige Werte ergeben haben. Darauf soll später noch eingegangen werden.

Bei einigen unserer Versuchstiere untersuchten wir vor und nach der Injektion einer letalen Dosis von Natriumzitrat den Kalziumgehalt des Blutes nach der Methode von Cramer-Tisdall.

V. Kalziumgehalt des Blutes nach intravenöser Injektion einer letalen Dosis von Natriumzitrat:

a) Bei Kaninchen.

A. Kalziumgehalt	5 Min. vor der Injektion	10,3	mg-%
„	2 Min. nach der Injektion	9,1	„
B. „	5 Min. vor der Injektion	10,5	„
„	2 Min. nach der Injektion	9,0	„
C. „	5 Min. vor der Injektion	10,7	„
„	2 Min. nach der Injektion	9,1	„

b) Bei Hunden.

A. Kalziumgehalt	5 Min. vor der Injektion	11,2	mg-%
„	2 Min. nach der Injektion	9,5	„
B. „	5 Min. vor der Injektion	10,8	„
„	2 Min. nach der Injektion	9,4	„
C. „	5 Min. vor der Injektion	11,1	„
„	2 Min. nach der Injektion	9,0	„

Diese Untersuchungen ergeben demnach, daß nach intravenöser Injektion einer tödlichen Dosis von Natriumzitrat der Kalziumgehalt des Blutes bei Kaninchen durchschnittlich von 10,5 auf 9,1, bei Hunden von 11,0 auf 9,3 mg-% absinkt. Dieser Befund deckt sich mit den Angaben von Clark und Rosenthal. Das Zitrat beschlagnahmt also offenbar die freien Kalziumionen des Blutes, aber nicht wie das Oxalat auch das organisch gebundene Kalzium. Für diese Anschauung spricht auch die Beobachtung, daß Natriumzitrat im Gegensatz zu Oxalat mit verdünnten Kalziumlösungen keinen Niederschlag gibt, jedoch die Dissoziation der Kalksalze aufhebt; aus diesem Verhalten muß geschlossen werden, daß durch Zitrat (ebenso wie durch Fluorid) die Kalziumsalze des Plasmas nicht gefällt werden, sondern — wenigstens teilweise — in nichtionisierter Form (als Komplexsalze) in Lösung bleiben. In Übereinstimmung mit dieser Auffassung steht auch die Beobachtung von Sluiter, daß Zitratblut durch Zusatz von $MgCl_2$ zur Gerinnung gebracht wird, Oxalatblut dagegen nicht, wobei die Wirkung des Mg offenbar darin besteht, daß es beim Zitratblut imstande ist, das nur komplex gebundene Kalzium aus seinen Bindungen freizumachen, indem es sich an seine Stelle setzt.

Wurden den Versuchstieren mehrere Tage hindurch kleine Dosen Zitrat (0,08—0,1 g pro Kilogramm) injiziert, so zeigte das Blut eine erhebliche Zunahme seiner Gerinnungsfähigkeit, die so stark war, daß z. B. eine Untersuchung des Blutes auf seinen Kalziumgehalt unmöglich wurde, da das mit Aqua dest. stark verdünnte Blut noch gelierte. Diese Zunahme der Gerinnungsfähigkeit des Blutes nach kleinen Zitratdosen, die nach Rosenthal auf

einer Auflösung der Blutplättchen beruht, erklärt die klinischen Erfahrungen von Neuhof und Hirshfeld, Renaud und Juge u. a., die durch Zitratinjektionen heftige Blutungen beim Menschen zum Stillstand zu bringen vermochten.

Der Hypothese von Rosenthal schließen sich Higgins und Fisher an; sie beobachteten nach intramuskulärer Injektion von Natriumzitrat eine rasche Herabsetzung der Blutgerinnungszeit im Verlauf von 1—3 Stunden mit allmählicher Rückkehr zur Norm innerhalb 24—48 Stunden. Durch die Zerstörung der Blutplättchen soll eine Substanz frei werden, die den Gerinnungsvorgang beeinflußt. Fehlen die Blutplättchen (Purpura u. a.), so bleibt der Erfolg aus.

Diese Hypothese würde geeignet erscheinen, die verschiedenartige Wirkungsweise des Natriumzitrats zu erklären, das teils die Blutgerinnung verhindert, teils — bei Einhaltung besonderer Versuchsbedingungen — die Blutgerinnung beschleunigt. Wir haben daher den Einfluß des Natriumzitrats auf die Blutplättchen in einer besonderen Versuchsreihe untersucht. Nach der Fonioschen Methode wurden bei einer Anzahl von Patientinnen (teils gesunden, teils gynäkologisch erkrankten Frauen) Blutausstriche hergestellt und die Thrombozyten ausgezählt. (Über die Technik siehe Löwenstein[1]). Als Verdünnungsflüssigkeit des aus der Fingerbeere austretenden Bluttropfens wurde 1,25 % und 30 % Natriumzitratlösung in physiologischer Kochsalzlösung sowie zur Kontrolle 14,4 % Magnesiumsulfatlösung (nach der Originalvorschrift) benutzt. Da es uns nur auf Vergleichswerte ankam, wurden jedesmal nur die auf 1000 Erythrozyten gefundenen Thrombozyten festgestellt.

Tabelle I.

Bestimmung der Thrombozyten nach der Fonioschen Methode, unter Zusatz von Magnesiumsulfat oder verschieden prozentiger Natrium-citricum-Lösung.

Nr. der Fälle	Magnesiumsulfat 14,4 %	Natriumzitrat 1,25 %	Natriumzitrat 30 %
1	96	99	—
2	42	—	41
3	66	41	53
4	66	70	71
5	118	99	133
6	86	79	84
7	70	57	65
8	54	71	48

Aus diesen Untersuchungen läßt sich eine gesetzmäßige Änderung der Thrombozyten bei Einwirkung von Natriumzitratlösung nicht feststellen. Die Schwankungen, die sowohl eine Vermehrung als auch eine Verminderung erkennen lassen, dürften innerhalb der Fehlergrenzen liegen.

[1]) Dr. Löwenstein hat in dankenswerter Weise die Auszählungen vorgenommen.

Um sicher zu sein, daß diese Befunde nicht auf einer fehlerhaften Technik beruhen, insbesondere auch, um die in der Blutbahn selbst sich abspielenden Vorgänge nach intravenöser Natriumzitratinjektion zu erfassen, haben wir auch Tierversuche angestellt. Zwei Kaninchen wurde Natriumzitrat intravenös eingespritzt; vor und nach der Injektion wurden zur Thrombozytenzählung Magnesiumsulfatausstriche aus der rasierten Ohrhaut hergestellt.

Tabelle II.

Thrombozytenzahl vor der Injektion	Injektion von	Thrombozytenzahl nach der Injektion	Verhalten des Tieres
	Tier Nr. I, 3750 g		
56	16 ccm einer 5%igen Lösung (0,8 g)	70	Exitus unter Dyspnöe und Krämpfen nach wenigen Minuten
	Tier Nr. II, 3200 g		
45	2 ccm einer 30%igen Lösung (0,6 g)	53	Keine Reaktion
5 Min. später	dieselbe Dosis (0,6 g)	47	Ruhe
5 Min. später	6 ccm einer 5%igen Lösung (0,3 g)	56	Krämpfe, Dyspnöe, Erholung
10 Min. später	dieselbe Dosis (0,3 g)	61	Krämpfe, schwere Dyspnöe, Erholung

Auch die Tierversuche lassen eine gesetzmäßige Verminderung der Thrombozyten nicht erkennen; man könnte im Gegenteil geneigt sein, eine Vermehrung anzunehmen. Wir möchten allerdings eher vermuten, daß diese Vermehrung nur eine scheinbare ist, da nach unseren Erfahrungen die Beimengung von Natr. citr. zum Blut die Thrombozyten besser konserviert und ihren Zerfall aufhält. Beim Vergleich unserer vom Menschen gewonnenen Ausstriche, die teils nach Zusatz von Magn. sulfuric., teils Natr. citr. hergestellt waren, fiel uns wiederholt auf, daß im Zitratpräparat die Thrombozyten viel besser zu erkennen und zum Teil besser erhalten waren, als im Magnesiumsulfatpräparat.

Nach diesen Versuchen erscheint uns die Theorie von Rosenthal unhaltbar. Eine Erklärung für die gesteigerte Blutgerinnung durch wiederholte kleine Gaben von Natr. citr. können wir vorläufig nicht geben. In diesem Zusammenhang sei erwähnt, daß Renaud und Juge die blutstillende Wirkung einer Natriumzitratinjektion auf eine Veränderung der Vasomotoren sowie auf die Begünstigung der Bildung von Thromben im blutenden Gewebe zurückführen.

Bei unseren bisher geschilderten Versuchen wurde den Versuchstieren eine reine Natriumzitratlösung injiziert. Bei einer Bluttransfusion bestehen aber andere Bedingungen insofern, als das hierbei verwandte Natrium citricum

nicht in reiner Form, sondern vermischt mit dem Spenderblut zugeführt wird. Es erhebt sich also die Frage, ob die ganze, dabei verwendete Zitratmenge auf den Organismus des Empfängers einwirkt. Wir stellten daher folgende Versuche an: Es wurden einem Tier (Kaninchen, Hund) aus der Vena jugularis 10—20 ccm Blut entnommen, die in eine für dieses Tier tödliche Dosis Zitratlösung eingebracht wurden. Dieses, durch Zitrat ungerinnbar gewordene Blut wurde nach 2 Minuten dem Versuchstier intravenös injiziert. Hierbei traten zwar toxische Symptome (Krämpfe, Dyspnoe) auf; nachdem diese jedoch binnen $^1/_2$ Minute verschwunden waren, zeigten die Tiere wieder ein normales Verhalten. Aus diesen Befunden kann gefolgert werden, daß nicht die ganze dem Spenderblut zugesetzte Zitratmenge auf den Empfängerorganismus schädigend einwirkt; ein Teil von ihr wird vom Spenderblut adsorbiert und hierdurch unwirksam gemacht.

Bezüglich der Wirkungen des Zitrats auf Atmung, Blutdruck, Peristaltik, Diurese u. a. sei hier nur auf die einschlägige Literatur verwiesen. Dagegen ist es erwähnenswert, daß nur reines Trinatriumzitrat bei Bluttransfusionen verwendet werden darf. So zeigte sich z. B. bei Zusatz von gleichen Mengen Mono- bzw. Trinatriumzitrat (je 1 ccm einer 20%igen Lösung zu 5 ccm Blut), daß mit Trinatriumzitrat versetztes Blut nach 6 Stunden noch nicht geronnen ist und eine hellrote Farbe aufweist, während bei Zusatz der gleichen Menge Mononatriumzitrat das Blut in kleinen Gerinnseln koaguliert und eine schokoladenbraune Färbung annimmt (Säurewirkung, Bildung von Hämin). Mit dieser Erfahrung bestätigen wir die Angaben von v. Wolff.

Es ist nun die Frage zu erörtern, ob der Zusatz von Natriumzitrat eine Änderung in den Immunitätsreaktionen des Blutes sowie Schädigungen der Blutelemente verursachen kann.

Beim Zustandekommen von schockartigen Symptomen ist die Verringerung bzw. das Verschwinden der Komplementfunktion bedeutungsvoll. Vom Natriumzitrat wissen wir, daß es diese Funktion in dem Sinne beeinflußt, daß die Bindung von Komplement an Blut und hämolytische Immunkörper und dadurch das Auftreten von Hämolyse verhindert wird (Gengou). Ferner findet nach Lewisohn eine Hemmung der Phagozytose bei Zusatz von Natrium citricum zum Blut nicht statt. Jeaubrau gibt an, daß Antikörper und endokrine Stoffe nicht zerstört werden. Nach den Untersuchungen von Cook führt die intravenöse Injektion von Natriumzitrat bei Kaninchen gegenüber den Kontrolltieren zu einer schnelleren und intensiveren Präzipitinbildung und erheblicher Verstärkung der Antikörperbildung, während die intravenöse Injektion von $CaCl_2$ diese Vorgänge stark hemmt. Auch auf die bakterizide Kraft des Blutes hat Zitrat nach den Untersuchungen von Walsh und Harmsworth keinen hemmenden Einfluß.

In der Literatur findet sich wiederholt die Angabe, daß durch eine Zitrat-

blutinfusion die Blutelemente geschädigt werden: Die Resistenz der Blutkörperchen werde vermindert, rote Blutkörperchen und die Blutplättchen würden geschädigt (Hempel, Beck). Diese Behauptungen sind nicht unwidersprochen geblieben. Jeaubrau berichtet, daß der Blutgefrierpunkt, der Viskositätskoeffizient und die respiratorische Fähigkeit der roten Blutkörperchen normal bleibe. Clairmont und M. A. Müller geben neuerdings an, daß Natrium citricum die gesamten Blutelemente nicht verändert, falls eine isotonische (4%ige) Lösung infundiert wird; Lewisohn lehnt einen Zerfall der roten Blutkörperchen ab; H. Opitz hat bei seinen Untersuchungen die Resistenz der Erythrozyten keineswegs herabgesetzt gefunden; Torii fand bei seinen Versuchen die Zahl der roten Blutkörperchen, auch bei Zufuhr von toxischen Dosen, nicht wesentlich verändert.

Aus diesen Untersuchungen und Erfahrungen ergibt sich also kein Anhaltspunkt für die Annahme, daß die immunisatorischen Prozesse im Organismus durch Natriumzitrat gehemmt werden; die vorliegenden Beobachtungen sprechen eher für eine günstige Beeinflussung. Weiter ist ein schädigender Einfluß auf die Blutelemente keineswegs erwiesen.

Normet führt die giftige Wirkung großer Zitratdosen auf eine Exzitation des sympathischen Nervensystems zurück. Diese exzitierende Wirkung werde nicht durch das Zitration, sondern durch das Natriumion verursacht: das elektrisch negative Säureradikal werde durch die Blutkolloide adsorbiert, dabei werde das Natriumion frei, das dann seinerseits andere positive Ionen des Gewebes verdränge. — Gegen diese Theorie sind folgende Bedenken einzuwenden: Es ist nicht bekannt, daß die Injektion von Natriumionen in Konzentrationen, wie sie bei der Injektion toxischer Dosen Natriumzitrat zur Verwendung kommen, zu Erregungszuständen des vegetativen Nervensystems führt. Ferner sind die Kolloide des Blutes und der Gewebe intra vitam negativ aufgeladen, so daß sie, wenn die Adsorption auf Grund elektrischer Vorgänge sich vollziehen würde, in erster Linie das Natrium adsorbieren würden; verläuft die Adsorption aber nicht nach den Regeln der polaren Adsorption, so kann es sich nur um eine Austauschadsorption auf Grund chemischer Affinitäten handeln, bei der das Zitration ein anderes negatives Ion verdrängt, das dann seinerseits wieder vom Natriumion des Zitrats gebunden wird.

Wir haben ausführlich die Möglichkeit einer Schädigung des Organismus bei Injektion von Natrium citricum erörtert, weil nur durch Klarstellung dieser Frage die Basis für eine gefahrlose therapeutische Anwendung des Natrium citricum bei Bluttransfusionen geschaffen wird. Unsere Tierversuche und Literaturstudien erwiesen erneut, daß Natrium citricum nur in gewissen Dosen gefährlich und unter Umständen auch tödlich wirken kann, daß aber bei Vermeidung der toxischen Dosen eine Zitratbluttransfusion ungefährlich ist und daß das transfundierte Blut sowohl als auch das Blut des

Empfängers keine ungünstigen Veränderungen erleidet. Bei der praktischen Ausführung einer Zitratbluttransfusion kommt es daher darauf an, ungefährliche Zitratdosen zu verwenden, d. h. unterhalb der toxischen Dosis zu bleiben. (Daß bei Überdosierung beim Menschen lebensbedrohliche Erscheinungen auftreten können, beweist der Fall von Skinner, bei dem anstatt 3%iger Natriumzitratlösung fälschlicherweise 30%ige verwandt worden war!) Anderseits ist zur Verhinderung der Gerinnung des Spenderblutes eine bestimmte Menge Natrium citricum erforderlich. Diese therapeutische Breite des Natrium citricum muß bekannt sein und innegehalten werden; dann liegt kein zwingender Grund vor, auf seine Verwendung bei der Bluttransfusion zu verzichten (Nürnberger).

Nürnberger hat in genauen Untersuchungen festgestellt, daß bereits 0,2 g Natrium citricum genügen, um 100 ccm Blut vor der Gerinnung zu schützen. Diese Menge stellt ein Minimum dar; 0,175 g verhindern die Gerinnung nicht mehr. Im praktischen Gebrauch wird es daher zweckmäßig sein, die Natriumzitratkonzentration etwas höher zu wählen, da bei der Bereitung der Spenderblutlösung leicht etwas zu viel Blut verwendet werden kann.

Die toxische Dosis von Natriumzitrat beträgt nach unseren Versuchsergebnissen, die als sehr niedrige Werte im Vergleich zu den Resultaten anderer Autoren anzusehen sind, für das Kaninchen 0,13 g pro kg, für den Hund 0,2 g. Unter Zugrundelegung dieser Dosen würde für eine 50 kg schwere Person die toxische Dosis 6,5—10 g Natrium citricum betragen. Nürnberger, der seinen Berechnungen einen Versuch an einem Hund zugrunde legte, wobei die verwandte Natriumzitratmenge nicht genau bekannt war, errechnete als tödliche Dosis für einen Menschen von 65 kg 11—15 g Natrium citricum. Unsere Berechnung stellt demnach eine sehr geringe Dosis dar. Das beweisen auch die Versuche von Renaud und Juge, die zur Bekämpfung von verschiedenen Blutungen beim Menschen Natriumzitratlösung intravenös injizierten; sie verwandten 10—25 ccm einer 30%igen Lösung, d. h. 3—7,5 g. Pasti berichtet über ähnliche Versuche; er ging in seiner Dosis bis zu 9 g, die er allerdings intramuskulär einspritzte.

Wie hoch sind nun die in der Praxis tatsächlich verwandten Natriumzitratmengen bei einer Bluttransfusion? Nach der Bummschen Vorschrift werden 500 ccm Blut mit 200—300 ccm 1%iger Natrium-citricum-Lösung in 0,9%iger Kochsalzlösung vermischt, d. h. auf 500 ccm Blut werden 2—3 g Natrium citricum gegeben. Bei Überleitung von 1000 ccm Blut (= 1400—1600 ccm Flüssigkeit) erhält der Empfänger 4—6 g Natrium citricum. Erst bei dieser recht großen Transfusionsmenge wird im Höchstfalle die von uns errechnete toxische Minimaldosis erreicht.

Döderlein verwendet ein Gemisch von 300 ccm Blut + 200 ccm

1%iger Natrium-citricum-Lösung, d. h. auf 300 ccm Blut 2 g, auf 1000 ccm Blut 6,7 g Natriumzitrat. Andere Autoren verwenden geringere Dosen.

An der Stoeckelschen Klinik besteht folgende Vorschrift: 250 ccm Normosallösung werden mit 25 ccm einer 5%igen Natrium-citricum-Lösung vermischt und dazu 250 ccm Spenderblut hinzugefügt. Der Empfänger erhält daher mit 1000 ccm Blut (2000 ccm Flüssigkeit) 5 g Natrium citricum, eine Dosis, die noch unter der toxischen Minimaldosis liegt und daher sicher ungefährlich ist.

In den meisten Fällen genügt es, bis zu 1 Liter Spenderblut überzuleiten, um eine lebensgefährliche Anämie erfolgreich zu bekämpfen. Die meisten Autoren empfehlen 500—800 ccm (Stickel u. a.). Mit dem Blut wird gleichzeitig eine beträchtliche Menge Verdünnungsflüssigkeit (Kochsalzlösung oder besser Normosallösung) dem Empfänger einverleibt; sie verstärkt selbstverständlich den therapeutischen Effekt, das Blutgefäßsystem wieder aufzufüllen. Für den Fall, daß man weniger Verdünnungsflüssigkeit, dafür aber mehr Blut zu transfundieren wünscht, d. h. also, daß der Empfänger mehr als 5—6 g Natrium citricum erhält, besteht wohl zunächst auch noch keine Gefahr; denn unsere Versuche zeigen, daß ein Teil des Natrium citricum, das dem Blut zu seiner Ungerinnbarmachung zugesetzt wird, für den Empfänger unschädlich gemacht wird. Die „toxische Minimaldosis", die wir für den Menschen errechnet haben, gilt nur für den Fall, daß eine reine Lösung von Natrium citricum, wie im Tierversuch, dem Menschen injiziert wird. Da aber die angewendete Konzentration, wie wir oben ausführten, für die Giftigkeit des Zitrats bedeutungsvoll ist, so empfiehlt es sich mehr, bei der Vornahme von Zitratbluttransfusionen niedrige Zitratkonzentrationen (eventuell durch Hinzufügen von Normosallösung zum Spenderblut) zu verwenden. Bei Einhaltung dieser Vorsichtsmaßnahmen ist sowohl auf Grund der Tierexperimente und Beobachtungen am Menschen als auch auf Grund theoretischer Überlegungen eine Gefahr für den Empfänger nicht zu befürchten.

Es bleibt zum Schluß noch die Frage, ob nach starken Blutverlusten die Verträglichkeit des Natrium citricum geändert ist (G. H. Schneider). Darüber liegen experimentelle Untersuchungen von Joannides und Cameron vor. Diese Autoren konnten feststellen, daß eine Ausblutung die Giftigkeit des Natriumzitrats nicht erhöht; dagegen beobachteten sie, daß bereits eine 0,2%ige Zitratlösung genügt, um den Blutdruck und die Lebenstätigkeit ansteigen zu lassen; auf Grund dieser Erfolge empfehlen die beiden Autoren die Zitratblutgabe auch für den Menschen.

Zusammenfassend kommen wir zu folgenden Schlußfolgerungen:

1. Todesfälle nach Natriumzitrattransfusionen, die auf Beimengung von Natriumzitrat zum Spenderblut zurückzuführen wären, sind bisher nicht sicher bekannt, soweit es sich nicht um grobe Irrtümer (unbeabsichtigte Überdosierung) handelte. Die in der Literatur

niedergelegten Todesfälle beruhen auf Unstimmigkeit des Empfänger- und Spenderblutes, sowie auf Giftwirkung des transfundierten defibrinierten oder alten Blutes.

2. Die toxische Minimaldosis von Natrium citricum errechnet sich nach unseren Versuchen für das Kaninchen auf 0,13 g pro kg Gewicht, für den Hund auf 0,2 g pro kg. Die Dosis letalis liegt um fast 50 % höher. Bei der Giftwirkung spielt die im Blut erreichte Konzentration von Natrium citricum eine bedeutungsvolle Rolle. Je langsamer injiziert wird, je geringer die Konzentration der Injektionsflüssigkeit von Natrium citricum ist, umso geringer ist die Toxizität.

3. Bei der Transfusion des Natriumzitratblutes wird ein Teil des Natrium citricum vom Spenderblut unwirksam gemacht; in eigenen Versuchen stellten wir fest, daß unter diesen Bedingungen die sonst letale Natriumzitratdosis nicht mehr den Tod herbeiführt.

4. Kleine Dosen Natrium citricum, mehrere Tage hindurch wiederholt injiziert, erhöhen die Gerinnungsfähigkeit des Blutes. Zerfall von Thrombozyten scheint dabei keine Rolle zu spielen.

5. Nach intravenösen Injektionen einer tödlichen Dosis von Natrium citricum sinkt der Kalziumgehalt des Blutes beim Kaninchen von 10,5 auf 9,1 mg-%, beim Hund von 11,0 auf 9,3 mg-%. Das Zitrat beschlagnahmt offenbar die freien Kalziumionen des Blutes, nicht aber das organisch gebundene Kalzium.

6. Auf eine Person von 50 kg Gewicht umgerechnet, ergibt sich eine toxische Minimaldosis für den Menschen von 6,5—10 g Natrium citricum. Diese Dosis ist, wie klinische Erfahrungen zeigen, ohne Schäden vertragen worden.

7. Bei der praktischen Ausführung der Bluttransfusion mit Natrium citricum kommen auf 1 Liter Blut 5, auf 2 Liter 10 g Natrium citricum. (Methode: Klinik Stoeckel.)

8. Veränderungen der immunisatorischen Vorgänge im Blut sowie der Blutelemente sind bei Natriumzitrattransfusionen, soweit die angegebenen Dosen nicht überschritten werden, nicht zu befürchten.

9. Die Transfusion von Natriumzitratblut ist ungefährlich unter der Voraussetzung, daß eine Überdosierung vermieden wird.

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THE EFFECT OF CERTAIN DRUGS ON GUINEA PIGS POISONED BY LETHAL DOSES OF LEAD

by

Momcilo St. Mokranjac, Sava Radmic and Danilo Soldatovic

The action of penicillin, PAS, BAL, Ca-EDTA, sodium citrate on guinea pigs poisoned with lethal doses of lead has been carried out. It has been found that under the given experimental conditions sodium citrate proves to be the only real antidote. The other drugs did not reduce but rather increased the mortality of poisoned animals.

During our extended studies of activation of lead by drugs extensively used in contemporary therapies for various illnesses, we have presently discovered that penicillin (1, 2, 3) as well as some other popular antibiotics (4) caused a stronger or weaker activation of lead. We emphasized in these papers that this fact may have the following significance:

1. The application of these antibiotics to victims of saturnism can lead to an abrupt activation of the already deposited lead which can have adverse consequences for the health of these patients.

2. The controlled activation of lead by penicillin and other antibiotics may eventually serve as a means for a gradual elimination of the already deposited lead which could remove the potential danger due to the existence of larger deposits of lead within the organism.

Our past works (1-4) dealt with the activity of penicillin and other antibiotics in experimental animals as well as in persons exposed to the conditions of a chronic lead poisoning. The present investigation studied the action of penicillin and PAS in experimental animals poisoned by lethal doses of lead for which there are no data in the literature. This was compared with the action of certain best known and recognized antidotes against lead poisoning, i.e. with EDTA and sodium citrate.

As the most interesting basis for our investigation we found the Moeshlin-Schechterman paper (5) describing the results of a comparative investigation of the action of BAL and sodium citrate in guinea pigs poisoned by lethal doses of lead as well as another Moeschlin paper (6) which describes the action of calcium-ethylenediamine-tetraacetate (Ca-EDTA), BAL, and sodium citrate in victims of saturnism. On the basis of these works (5,6) the authors have concluded that in acute lead poisoning as well as in the case of lethal doses the most appropriate antidote is sodium citrate. On the basis of his experience with the use of Ca-EDTA in men, Moeschlin (6) came to the conclusion which contradicts the now almost universally accepted viewpoint according to which Ca-EDTA is presently the best, most efficient, and a comparatively harmless antidote in lead poisoning. Moeschlin quoted even certain cases of deaths due to the use of Ca-EDTA leading to a conclusion (6) that Ca-EDTA should be withdrawn from the therapy until a more detailed investigation of its effects as a lead antidote is carried out.

Experimental Part

Preliminary Investigations. -- We, first of all, reproduced the Moeschlin-Schechterman experiments on guinea pigs consisting in the following: groups of 20 guinea pigs each (I) have been poisoned the first day with 120 mg/kg of lead nitrate given intraperitoneally, followed on the fifth and eighth day by additional 60 mh/kg doses each.

A group of guinea pigs (II) treated with sodium citrate has received daily from 1st to 15th day 0.4 mg/kg, and from 15th to 21st day 0.2 mg/kg of lead

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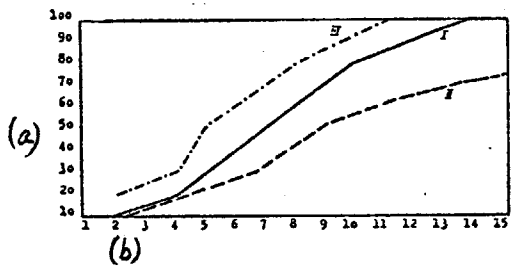
nitrate. Sodium nitrate was given per os.

The group of guinea pigs (III) treated with BAL received daily from the 1st to 4th day of the poisoning 3 mg/kg of BAL four times a day and from the 5th to 14th day 3 mg/kg of lead nitrate twice a day.

These preliminary investigations supplied results (Diagram 1) which within the limits of possible accuracy agreed fully with the Moeschlin and Schtechterman results.

From the mortality curve of the guinea pigs which received only lead in lethal doses and of those which were treated with sodium citrate and BAL one can clearly see that the use of sodium citrate reduces significantly the mortality of guinea pigs and that certain number of the animals so treated even survives the poisoning. (In our experiment the number was 30% while Moeschlin found 20%). The use of BAL even increases the mortality of animals. Moeschlin and Schechterman came to the same conclusions.

Diagram 1



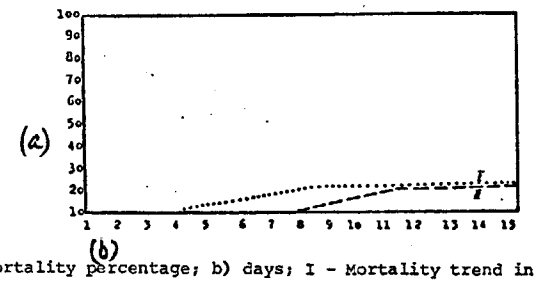
a) - Mortality percentage; b) Days. I = Mortality trend in guinea pigs of the control group; II - Mortality trend in guinea pigs of the group receiving sodium citrate; III - Mortality trend in guinea pigs receiving BAL.

Own Investigations. -- We attempted to establish through our investigations the degree to which the life span of guinea pigs poisoned with lethal doses of lead may be lengthened or shortened by the action of penicillin or PAS as compared with the effects of Ca-EDTA, BAL, and sodium citrate, all of

this, of course, relative to the life span of guinea pigs poisoned with the same lethal doses of lead but without any treatment.

As a preliminary to these experiments, we studied the effect of penicillin, BAL, PAS, Ca-EDTA, and sodium citrate, alone, on the organism of guinea pigs exposing them to the same amount of the above mentioned drugs as used during the experiments with the poisoned guinea pigs. BAL in amounts given (and which were the same as those used by Moeschlin in his experiments) caused death in 10% of the guinea pigs on the fourth day and another 10% on the eighth day of the application. (Moeschlin in his experiments did not test the action of BAL). With Ca-EDTA (II) he caused death in 10% of the guinea pigs on the eighth day and in 10% on the eleventh day. Penicillin, sodium citrate, and PAS did not cause any changes.

Diagram 2



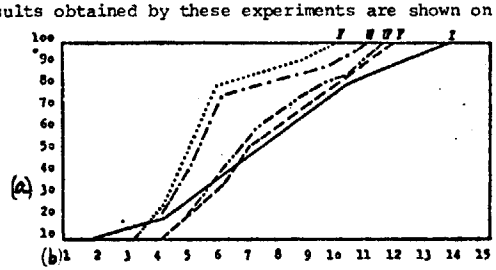
a) - Mortality percentage; b) days; I - Mortality trend in guinea pigs of the group which received BAL; II - Mortality trend in guinea pigs of the group which received Ca-EDTA.

Following these investigations we proceeded to experiment with animals poisoned with lethal doses of lead. The doses for lead poisoning of guinea pigs were the same as those utilized in experiments carried out by Moeschlin (6) while the quantities of BAL and sodium citrate given to the animals were likewise the same as those administered by Moeschlin in order to be able to compare our results with his. As far as Ca-EDTA is concerned, which did not figure in Moeschlin's experiments, we used as a criterion for the determina-

tion of the quantities given the amounts of this drug which are used for the treatment of men, recalculated, of course, per kilogram body weight of the animal. In the case of penicillin and PAS the criterion for the determination of the doses utilized in our experiment were the doses which were within the limits used in the treatment of men -- again, of course recalculated per kilogram body weight of animals.

Experiments with Penicillin. -- guinea pigs treated with penicillin were divided into five groups of 10 guinea pigs each. The first group (I) served as the control receiving little doses of lead. The next four groups of five (sic) guinea pigs each were again poisoned with the same amounts but for ten days during the experiment they received daily varying amounts of penicillin. The second group (II) received 25,000 IU of penicillin per kilogram of weight, the third (III) got 10,000 IU daily, the fourth 5,000 IU, and the fifth (V) 1,000 IU of penicillin per day. Penicillin was administered intramuscularly. The results obtained by these experiments are shown on Diagram 3.

Diagram 3

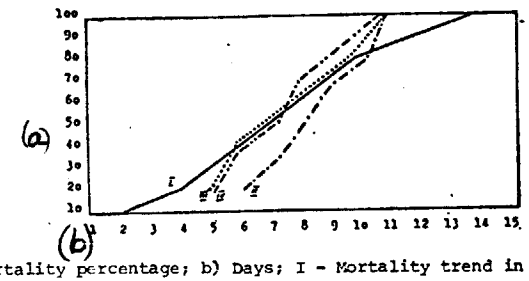


a) Mortality percentage; b) Days; I - Mortality trend in guinea pigs of the control; II - Mortality trend in guinea pigs of the group which received 25,000 IU of penicillin daily; III - Mortality trend in guinea pigs of the troup which received 10,000 IU penicillin daily; IV - Mortality trend in guinea pigs of the group which received 5,000 IU of penicillin daily; V - Mortality trend in guinea pigs of the group which received 1,000 IU of penicillin daily.

Experiments with the P-Aminosalicilic Acid (PAS). -- Guinea pigs treated

with PAS have been likewise divided into five groups of ten guinea pigs each. The first group (I) was the control one and received only the lethal doses of lead. The subsequent four (sic) groups were poisoned with the same amount of lead but received during the experiment various amounts of PAS, i.e. 0.05 g daily (II), 0.2 g daily (III), and 0.5 g daily (IV) per kg of body weight. PAS was administered daily over a span of 10 days starting from the first day of the poisoning and given per os. The results of these experiments are shown on Diagram 4 (I-IV).

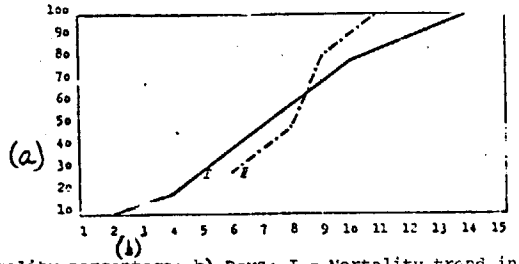
Diagram 4



a) Mortality percentage; b) Days; I - Mortality trend in guinea pigs of the control group; II - Mortality trend in guinea pigs of the group which received 0.05 g of PAS daily; III - Mortality trend in guinea pigs of the group which received 0.2 g of PAS daily; IV - Mortality trend in guinea pigs of the group which received 0.5 g of PAS daily.

Experiments with Calcium-Ethylenediamine-Tetraacetate (Ca-EDTA). -- Guinea pigs treated with this preparation were poisoned with the same amount of lead as the ones in the previous experiments and then they received daily the drug over a period of ten days in the amount of 25 mg per kg of weight. Ca-EDTA was given intravenously. The control group received only lead. Results are shown Diagram 5.

Diagram 5



a) Mortality percentage; b) Days; I - Mortality trend in guinea pigs of the control group; II - Mortality trend in guinea pigs of the group receiving Ca-EDTA.

Discussion and Conclusions

As can be seen from the graphs summarizing our results, all the drugs used can be divided into three groups in order to establish their effect on the animals poisoned with lethal doses of lead. The first group contains sodium citrate whose effect is absolutely positive. It is the only one for which one can say that it acted in this case as an efficient antidote in poisonings with lethal doses of lead. When treated with sodium citrate, about 30% of guinea pigs survived and could be considered cured.

The second group comprises Ca-EDTA and PAS whose effects are quite similar. All the guinea pigs treated with EDTA and PAS stayed alive for five or six days, from the beginning of the poisoning. After the sixth day deaths started quite abruptly and continued rather uniformly up to the eleventh day when the last guinea pigs from these two groups died.

The third group comprises penicillin and BAL. They also act in a similar way. In the case of BAL deaths start on the second day while in the case of penicillin (with higher doses) on the fourth day. Subsequently the mortality increases very rapidly up to the sixth or seventh day. After that the mortality curve has a somewhat lesser slope up to the tenth or twelfth day when we witnessed the expiration of even the last guinea pig.

If we finally compare the effect of all the tested drugs, we can conclude that PAS and Ca-EDTA act to certain degree favorably in extending the life of guinea pigs acutely poisoned with lethal doses of lead but only during the first days following the poisoning. Later, their action is only negative, i.e. they increase the mortality of the treated animals. Penicillin and BAL act very adversely and, to a certain degree, increase the mortality precisely during the first days following the introduction of lead. Only sodium citrate decreases the mortality even during the first days following the introduction of lead as well as after the acute phase.

Our experiments dealt with the study of the effect of these drugs during acute poisoning with lethal doses of lead. In case of chronic poisoning, especially those caused by continuous introduction of very small amounts of lead, the conclusions may come out different. Namely, the action of sodium citrate may be explained (5, 7) by the conversion of the toxic salts of lead into a nontoxic complex which remains stored in the organism, because during the administration of citrate there was no increase in the secretion of lead present (8). However, a substantial deposit of lead within the organism constitutes surely a potential danger for the victims of saturnism. In case of chronic poisoning, it is therefore very useful to administer substances which will slowly and in a controlled way eliminate lead from the organism.

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Delovanje nekih lekova na zamorce trovane letalnim dozama olova

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Ispitano je delovanje penicilina, PAS-a, BAL-a, Ca-EDTA i natrijum-citrata na zamorcima, koji su trovani letalnim dozama olova. Konstatovano je da u navedenim eksperimentalnim uslovima, kao realan antidot deluje samo natrijumcitrat. Ostali lekovi ne samo da smanjuju, već i povećavaju smrtnost trovanih životinja.

On a étudé l'action de la pénicilline, du PAS, du BAL, du Ca-EDTA et du citrate de soude sur les cobayes intoxiqués par les doses létales de plomb. On a constaté qu'il n'y a que le citrate de soude qui, dans les conditions expérimentales decrites, agit comme un antidote réel. Les autres médicaments agissent plutôt d'une façon négative, c'est à dire qu'ils augmentent la mortalité des animaux intoxiqués.

Baveći se već duže vremena pitanjem mobilizacije olova lekovima koji se danas mnogo primenjuju u savremenoj terapiji kod raznih oboljenja, utvrdili smo zasada da penicilin (1, 2, 3), kao i neki drugi mnogo upotrebljavani antibiotici (4) izazivaju jaču ili slabiju mobilizaciju olova. U tim smo radovima istakli da bi ova činjenica mogla imati sledeći značaj:

1. primena tih antibotika kod saturničara mogla bi dovesti do nagle mobilizacije već deponovanog olova, što bi moglo imati i štetne posledice po zdravlje saturničara;

2. kontrolisana mobilizacija olova penicilinom ili drugim antibioticima mogla bi eventualno poslužiti kao sredstvo za postepeno eliminisanje već deponovanog olova, čime bi se uklonila potencijalna opasnost postojanja većih depoa olova u organizmu.

Naši dosadašnji (1—4) radovi odnosili su se na delovanje penicilina i drugih antibiotika kod eksperimentalnih životinja, kao i kod ljudi, izloženih uslovima hroničnog trovanja olovom. U ovom radu ispitali smo delovanje penicilina i PAS-a kod eksperimentalnih životinja trovanih letalnim dozama olova, o čemu nema podataka u literaturi, i uporedili njihovo delovanje sa dejstvom nekih najpoznatijih i priznatih antidota za trovanja olovom, tj. sa EDTA i natrijum-citratom.

Kao baza za ova naša istraživanja učinio nam se naročito interesantan Moeschlinov i Schechtermanov (5) rad, u kome su izneti rezultati komparativnog ispitivanja delovanja BAL-a i natrijum-citrata kod zamoraca trovanih letalnim dozama olova, kao i drugi Moeschlinov rad (6) u kome je obrađeno delovanje kalcijum-etilendiamino-tetraacetata (Ca-EDTA), BAL-a i natrijum-citrata kod saturničara. Na osnovu tih radova (5, 6) autori su zaključili da je kod akutnih trovanja olovom, pa i kod letalnih doza, najpovoljniji antidot natrijum-citrat. A na osnovu svojih iskustava sa upotrebom Ca-EDTA kod ljudi, Moeschlin (6) je došao do jednog zaključka, koji je u suprotnosti sa danas skoro opšte prihvaćenim gledištem, po kome je Ca-EDTA zasada najbolji, najefikasniji i relativno bezopasan antidot kod trovanja olovom. Moeschlin je naveo i neke slučajeve letalnog završetka usled upotrebe Ca-EDTA, tako da čak (6) izražava mišlenje da bi Ca-EDTA trebalo isključiti iz terapije tako dugo, dok se detaljnije ne ispita njegovo dejstvo kao antidota na olovo.

Sodium citrate 263

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EKSPERIMENTALNI DEO

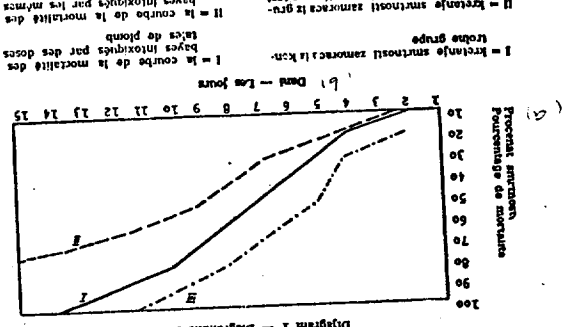
Pretходna ispitivanja. — Pre svoga započetog rada sam Moeschlinove i Schecheterman-ove eksperimente na zamorcima koji su se sastojali u sledećem: grupe od po 20 zamoraca (I) tretirane su prvog dana sa 120 mg/kg olovo-nitrata datog intraperitonealno, petog dana davano im je još 60 mg/kg i osmog dana 60 mg/kg.

Grupa zamoraca (II) tretirana natrijum-citratom dobivala je svakodnevno od 1.—15. dana po 0,4 mg/kg, a od 15.—21. dana po 0,2 mg/kg olovo-nitrata. Natrijum-citrat je davan per os.

Grupa zamoraca (III) koja je lečena BAL-om dobila je 1.—4. dana trošnja svakodnevno 4 puta dnevno po 3 mg/kg BAL-a, a od 5.—14. dana dva puta po 3 mg/kg olovo-nitrata svakoga dana.

Ova naša pretходna ispitivanja dala su nam rezultate (dijagr. 1), koji su se u granicama mogućе tačnosti potpuno slagali sa Moeschlinovim i Schecheterman-ovim rezultatima.

Iz dobivene krive smrtnosti zamoraca kojima je davano samo olovo u letalnim dozama i onih koji su lečeni natrijum-citratom i BAL-om vidi se jasno da upotreba natrijum citrata znatno smanjuje smrtnost zamoraca i da čak izvesan deo ovako lečenih životinja preživi ovo trovanje. (U našem eksperimentu 30%, a kod Moeschlina 20%). Upotrebom BAL-a smrtnost se životinja i povećava. Do tih zaključaka došli su i Moeschlin i Schecheterman.



Dijagram 1 — Diagramme 1

I = kretanje smrtnosti zamoraca iz kontrolne grupe

II = kretanje smrtnosti zamoraca iz grupe koja je primala natrijum citrat

III = kretanje smrtnosti zamoraca iz grupe koja je primala BAL

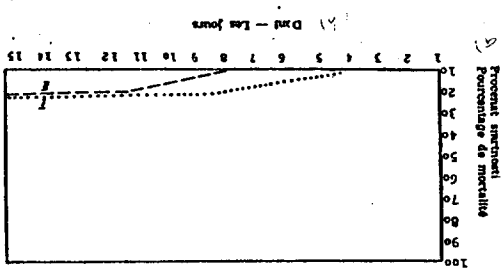
I = la courbe de la mortalité des cobayes intoxiqués par des doses létales de plomb

II = la courbe de la mortalité des cobayes intoxiqués par les mêmes doses de plomb, mais qui ont été traités par le citrate de soude

III = la courbe de la mortalité des cobayes intoxiqués par des doses létales de plomb, mais que ont été traités par le BAL

Dopstvena istraživanja. — Našim smo istraživanjima htjeli da utvrdimo u kojoj će se meri život zamoraca, trovanih letalnim dozama olova, produžiti ili skratiti dotovanjem penicilina i PAS-a u poređenju sa dotovanjem Ca-EDTA, BAL-a i natrijum-citrata, a sve naravno u odnosu na dužinu života zamoraca trovanih istim letalnim dozama olova, ali bez ikakvog lečenja.

Pre nego što smo prešli na ove eksperimente, ispitali smo dotovanje samog penicilina, BAL-a, PAS-a, Ca-EDTA i natrijum-citrata na organizam zamoraca, dajući im one količine pomenutih lekova koje smo upotrebljavali i u samom eksperimentu sa trovanim zamorcima. BAL je u količinama koje smo mi davali (a koje je upotrebljavao i Moeschlin u svojim eksperimentima) proizrokovao smrt 10% zamoraca četvrtog dana i još 10% osmog dana davanja. (Moeschlin u svojim eksperimentima nije vršio probe dotovanja samog BAL-a). Ca-EDTA (II) proizrokovao je smrt 10% zamoraca osmoga dana i 10% jedanaestoga dana. Penicilin, natrijum-citrat i PAS nisu proizrokovali nikakve promene.



Dijagram 2 — Diagramme 2

I = kretanje smrtnosti zamoraca iz grupe koja je primala po 5.000 IJ penicilina

II = kretanje smrtnosti zamoraca iz grupe koja je primala po 1.000 IJ penicilina

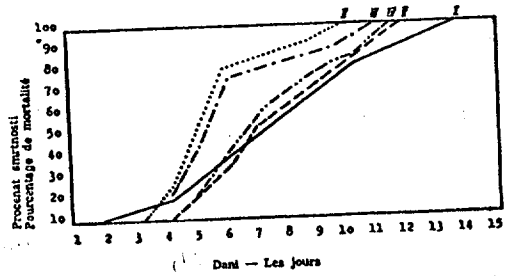
I = la courbe de la mortalité des cobayes intoxiqués par des doses létales de plomb et traités par les 5.000 UI de pénicilline par jour et par kg

II = la courbe de la mortalité des cobayes intoxiqués par des doses létales de plomb et traités par les 1.000 UI de pénicilline par jour et par kg

Ca-EDTA, koji u svom eksperimentu Moeschlin nije ispitivao, mi smo uzeli kao merilo za određivanje davanih količina one količine Ca-EDTA koje se upotrebljavaju za lečenje ljudi, naravno sračunato na kilogram telesne težine životinje. Što se tiče penicilina i PAS-a, tu smo kao merilo za određivanje doza koje smo primenili u ovim našim eksperimentima, uzeli doze koje su bile u granicama onih količina što se upotrebljavaju u terapiji ljudi — naravno opet sračunato na kilogram telesne težine životinje.

Eksperimenti sa penicilinom. — Zamorce koje smo tretirali penicilinom podelili smo u pet grupa od po deset zamoraca. Prva grupa (I) je služila kao kontrolna, i njima su davane letalne doze olova. Sledeće četiri grupe od po pet zamoraca opet su trovane istim količinama, ali su u toku eksperimenta primale deset dana svakodnevno različite količine penicilina. Druga grupa (II) je primala po 25.000 IJ penicilina na kg težine, treća (III) po 10.000 IJ, četvrta (IV) po 5.000 IJ i peta (V) 1.000 IJ penicilina. Penicilin je davan intramuskularno. Rezultati dobijeni ovim eksperimnetima pretstavljeni su na dijagr. 3.

Dijagram 3 — Diagramme 3



I = kretanje smrtnosti zamoraca iz kontrolne grupe

II = kretanje smrtnosti zamoraca iz grupe koja je primala po 25.000 IJ penicilina

III = kretanje smrtnosti zamoraca iz grupe koja je primala po 10.000 IJ penicilina

I = la courbe de la mortalité des cobayes du groupe de contrôle, qui sont intoxiqués par des doses létales de plomb

II = la courbe de la mortalité des cobayes intoxiqués par des doses létales de plomb et traités par les 25.000 UI de pénicilline par jour et par kg

III = la courbe de la mortalité des cobayes intoxiqués par des doses létales de plomb et traités par les 10.000 UI de pénicilline par jour et par kg

IV = kretanje smrtnosti zamoraca iz grupe koja je primala po 5.000 IJ penicilina

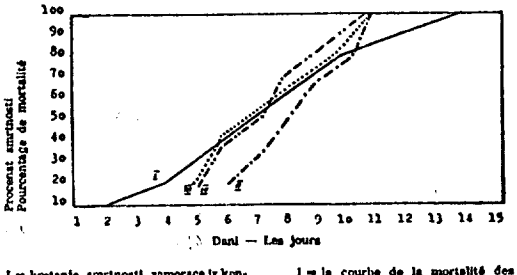
V = kretanje smrtnosti zamoraca iz grupe koja je primala po 1.000 IJ penicilina

IV = la courbe de la mortalité des cobayes intoxiqués par des doses létales de plomb et traités par les 5.000 UI de pénicilline par jour et par kg

V = la courbe de la mortalité des cobayes intoxiqués par des doses létales de plomb et traités par les 1.000 UI de pénicilline par jour et par kg

Eksperimenti sa p-aminosalicilnom kiselinom (PAS). — Zamorci tretirani PAS-om podeljeni su takođe na pet grupa od po deset zamoraca. Prva grupa (I) bila je kontrolna i njima su davane samo letalne doze olova. Sledeće četiri grupe trovane su istim količinama olova, ali su u toku eksperimenta primale različite količine PAS-a, i to po 0,05 g (II), 0,2 g (III) i 0,5 g (IV) na kg telesne težine. PAS je davan svakodnevno u toku 10 dana, počevši od prvog dana trovanja, i to per os. Rezultati dobijeni ovim eksperimentima pretstavljeni su na dijagr. 4 (I—IV).

Dijagram 4 — Diagramme 4



I = kretanje smrtnosti zamoraca iz kontrolne grupe

II = kretanje smrtnosti zamoraca iz grupe koja je primala po 0,05 g PAS-a

III = kretanje smrtnosti zamoraca iz grupe koja je primala po 0,2 g PAS-a

IV = kretanje smrtnosti zamoraca iz grupa koja je primala po 0,5 g PAS-a

I = la courbe de la mortalité des cobayes du groupe de contrôle, qui sont intoxiqués par des doses létales de plomb

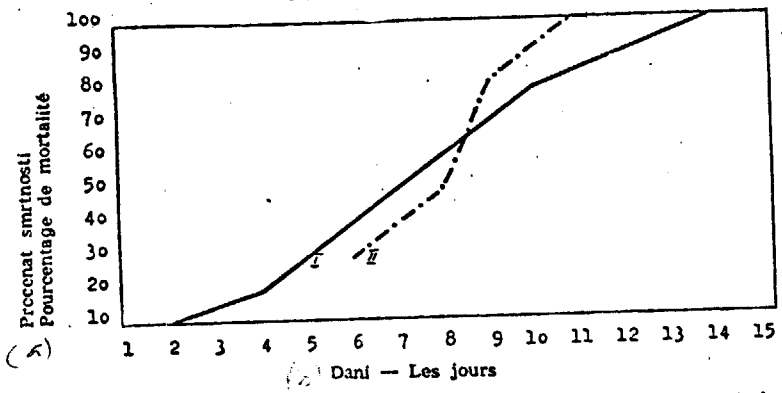
II = la courbe de la mortalité des cobayes intoxiqués par des doses létales de plomb et traités par 0,05 g de PAS par jour et par kg

III = la courbe de la mortalité des cobayes intoxiqués par des doses létales de plomb et traités par 0,2 g de PAS par jour et par kg

IV = la courbe de la mortalité des cobayes intoxiqués par des doses létales de plomb et traités par 0,5 g de PAS par jour et par kg

Eksperimenti sa kalcijum-etilendiaminotetraacetatom (Ca-EDTA). — Zamorci lečeni ovim preparatom trovani su istim količinama olova kao i u prethodnim eksperimentima, pa im je onda davan preparat svakodnevno u toku deset dana po 25 mg na kg težine. Ca-EDTA je davan intravenozno. Kontrolnoj grupi je davano samo olovo. Rezultati prikazani su na dijagramu 5.

Dijagram 5 — Diagramme 5



I = kretanje smrtnosti zamoraca iz kontrolne grupe

I = la courbe de la mortalité des cobayes du groupe de contrôle, qui sont intoxiqués par des doses létales de plomb

II = kretanje smrtnosti zamoraca iz grupe koja je primala Ca-EDTA

II = la courbe de la mortalité des cobayes intoxiqués par des doses létales de plomb et traités par 25 mg de Ca-EDTA par jour et par kg.

DISKUSIJA I ZAKLJUČAK

Kao što se iz grafičkih prikaza naših rezultata vidi, sve lekove koje smo davali da bi utvrdili njihovo delovanje na životinje trovane letalnim dozama olova, možemo podeliti na tri grupe: u prvoj grupi se nalazi natrijum-citrat čije je delovanje apsolutno pozitivno. Za njega se jedino može reći da u ovom slučaju deluje kao efikasan antidot kod trovanja letalnim dozama olova. Davanjem natrijum-citrata oko 30% zamoraca je preživelo trovanje i mogu se smatrati izlečenima.

U drugu grupu dolazi Ca-EDTA i PAS čije je delovanje vrlo slično. Svi zamorci lečeni EDTA i PAS-om ostali su u životu pet do šest dana od početka trovanja. Posle šestog dana umiranje je počelo prilično naglo i išlo dosta ravnomerno sve do jedanaestog dana kada su uginuli i poslednji zamorci iz ove dve grupe.

U treću grupu dolaze penicilin i BAL. I oni slično deluju. Umiranje počinje kod BAL-a drugog dana, a kod penicilina (kod većih doza) četvrtog dana. Zatim smrtnost naglo raste sve do šestog ili sedmog dana. Posle ovoga kriva smrtnosti ima nešto blaži porast sve do desetog ili dvanaestog dana kada dolazi do letalnog završetka i poslednjeg zamorca.

Ako na kraju poredimo delovanje svih iskušanih lekova, možemo zaključiti da PAS i Ca-EDTA u izvesnoj meri povoljno deluju na produženje života zamoraca akutno trovanih olovom samo u prvim danima posle prvog trovanja. Docnije je njihovo delovanje samo negativno, tj. povećava smrtnost lečenih životinja. Penicilin i BAL deluju vrlo nepovoljno i u znatnoj meri povećavaju smrtnost baš u prvim danima posle unošenja olova. Jedino natrijum-citrat smanjuje smrtnost već u prvim danima posle unošenja olova, kao i u danima kad je akutna faza već prošla.

U ovim našim eksperimentima radilo se o ispitivanju dejstva ovih lekova kod akutnih trovanja letalnim dozama olova. Kod hroničnih trovanja, naročito onih izazvanih stalnim unošenjem vrlo malih količina olova, možda bi zaključci bili drukčiji. Naime, delovanje se natrijum-citrata objašnjava (5, 7) pretvaranjem toksične soli olova u netoksični kompleks, koji ostaje deponovan u organizmu, pošto se kod davanja citrata ne konstatuje povećano izlučivanje olova (8). Međutim veći depo olova u organizmu pretstavlja sigurno potencijalnu opasnost za saturničare, i zato je kod hroničnih trovanja sigurno korisno davati sredstva koja će postepeno i kontrolisano izlučivati olovo iz organizma.

INSTITUT ZA TOKSIKOLOŠKU HEMIJU
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BEOGRAD

Primljeno: 8. XII. 1958

Action de certains médicaments sur les cobayes intoxiqués par des doses létales de sel de plomb

M. St. Mokranjac, S. Radmić et D. Soldatović

RÉSUMÉ

Dans leurs travaux antérieurs les auteurs ont déja fait l'étude sur la mobilisation du plomb produite sous l'effet du traitement par certains antibiotiques (1, 2, 3, 4) des animaux et des hommes soumis préalablement aux conditions d'intoxication chronique par le plomb. Dans le travail présent ils ont entrepris l'étude de l'action de la pénicilline et du PAS sur les animaux intoxiqués par des doses létales du plomb, et ils ont, en même temps, fait une étude comparative des effets que produisent dans les mêmes conditions d'intoxications, certains substances qui sont utilisées comme antidotes dans les intoxications par le plomb.

Pour avoir une base de comparaison dans leur étude ils ont repris d'abord l'expérience de Moeschlin et de Schechterman (5) dans laquelle ces auteurs ont étudié l'action du BAL et du citrate de soude sur les cobayes intoxiqués par des doses létales de plomb: les cobayes ont été d'abord intoxiqués en leur donnant, par voie intraperitonéale, 120 mg/kg du nitrate de plomb, le cinquième jour ont leur redonnait encore 60 mg/kg et le huitième jour encore 60 mg/kg.

Le groupe des animaux intoxiqués, mais traités par le citrate de soude, recevaient du 1er au 15ème jour 0,4 g/kg et du 15ème au 21ème jour 0,2 g/kg par jour du citrate de soude (diagr. 1/II).

Les animaux ainsi intoxiqués et traités par le BAL recevaient du premier au quatrième jour d'intoxications 4 fois par jour 3 mg/kg de BAL, et à partir du cinquième jour jusqu'au 14ème les animaux recevaient deux fois par jour 3 mg/kg de BAL (diagr. 1/III).

Dans cette expérience, qui consistait dans la reproduction de l'expérience de Moeschlin et de Schechterman, on a obtenu des résultats semblables à ceux des auters cités: par le traitement avec le BAL la mortalité des animaux n'a pas du tout diminué, mais au contraire a augmenté. Cependant chez les animaux traités par le

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Acta Pharmaceutica Jugoslavica, No. 2, 1960, Zagreb.

COULOMETRIC DETERMINATION OF SALTS

by

Kosta Nikolic and Radomir Popovic

A method for the coulometric determination of potassium and sodium salts has been proposed. These salts were titrated in the cathode compartment by means of electrical currents and the quantities of slats were deduced from the number of consumed Coulombs.

The Yugoslav Pharmacopoeia II as well as other pharmacopoeias contain prescriptions for the determination of potassium and sodium salts. These prescriptions are based on classical volumetric and gravimetric methods. In view of the distinguished qualities of the coulometric method, we attempted to apply this procedure to the determination of certain potassium and sodium salts which are required by the Yugoslav Pharmacopoeia. We determinated the following salts: potassium-sodium-tartarate, potassium-nitrate, potassium tartrate, potassium sulfate, sodium acetate, sodium citrate, sodium nitrate, and sodium sulfate. These were officinal salts, either as medicinal substances or reagents.

Being that these are all salts of alkaline metals, we were able to embark upon a coulometric determination only following an ion exchange. Utilizing a very acid cation exchanger "Merck" I, we carried out the ion exchange process described by the equation:

$$R - \overset{-}{SO_3}\overset{+}{H} + \overset{+}{Me}\ \overset{-}{Y} \rightleftharpoons R - \overset{-}{SO_3}\overset{+}{Me} + \overset{+}{H}\overset{-}{Y}$$

Me — ion of the alkaline metal

Since this exchange liberates an equivalent amount of hydrogen ions, the eluate contained a corresponding acid which generates the salt; we were able to titrate coulometrically this acid within the cathode compartment. The

amount of Coulombs of the current utilized permitted the calculation of the amount of salt which had to be determined.

All tested salts were well soluble in water as were the acids obtained following the ion exchange. Consequently, we used as solvent freshly redistilled water.

The final titration points were determined by appropriate indicators. Yugoslav Pharmacopoeia II recommends methyl-orange for the determination of the nitric and sulphuric acids. We did not find this suitable because of an unclear transition in color making the results of the determination non-nonreproducible. Since the USP and Internation Pharmacopoeia prescribe methil-red, we decided to use the latter indicator. It proved to be very suitable during all the determinations, for the change in color is much more pronounced. Since the sodium sulfate solution is neutral while the change in color occurs in acid medium (4.40 - 6.20), we had to add 1 ml 0.1 N of sulfuric acid to 100 ml of the sodium sulfate solution using it as catholyte. In view of a control test under the same conditions, the addition of acid did not affect the accuracy of the determination.

Experimental Part

Reagents and Chemicals:

a) The solution for the cathode compartment (catholyte): 142.05 g of sodium sulfate dissolved in water with a water addition up to 1,000 ml; to this is added 10 ml 0.1 N of sulfuric acid and all is mixed well.

b) The solution for the anode compartment (anolyte): 142.05 g of sodium sulfate is dissolved in water and the solution is brought up to 1,000 ml with additional water.

Indicators:

-- Methyl-red: 0.1 g is dissolved in 100 ml of 70% ethanol.

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-- Phenolphthalein: 1 g is dissolved in 70 ml of 70% ethanol.

-- Phenolphthalein: 1 g is dissolved in 70 ml of concentrated ethanol and the solution is then diluted with water up to 100 ml.

During the determination of the salt we utilized also a 0.01 N solution of sodium hydroxide which can be produced by coulometric procedure.

The ion exchange has been carried out by a very acid cation exchanger resin "Merck" I.

Instrumentation. -- The stable d.c. current was obtained from a selenium rectifier connected through a transformer and stabilizer to the commercial network. The current intensity was controlled by means of a variable resistor and transformer with a continuous voltage variation from 0 to 230 V. We used platinum electrodes. The cathode compartment was separated out by means of a container with porous bottom. To prevent the mixing of catholyte and anolyte, the bottom of this container was covered with agar-agar containing dissolved sodiumnitrate.

A precise milliammeter "Iskra" registered the intensity of the current.

The Preparation of the Ion Exchange Column. -- At the bottom of a glass tube 1 cm in diameter we put fiberglass and then filled the rest with the exchanger resin. To maintain the continuity of the column, it is advisable to likewise close the top of the column with a fiberglass tampon. The height of the column should be 12-14 cm. Prior to the filling of the column, the resin must swell in water for 24 hours.

Prior to the passage of the solution, the column must be activated by passing through it 25 ml of a 10% solution of hydrochloric acid and then rinsing it with distilled water down to a negative reaction to chlorides.

The Regeneration of the Ion Exchanger. -- Following each utilization, the exchanger is regenerated by passing 25 ml of the 10% solution of hydrochloric acid. Following the passage of this solution the column is rinsed with water down to a negative reaction to chlorides.

Procedure. -- The solution for coulometric titration was prepared in the following manner: 100 mg of the salt is measured off and dissolved in 10 ml of freshly boiled distilled water; the solution is then led through the column of the previously activated cation exchanger. The cup which held the solution is then rinsed with 5 ml of water which is then also led through the column. The column is rinsed with water until the volume of the solution which passed through the column is equal to 50 ml and is collected into a 50 ml measure positioned under the glass tube containing the ion exchanger. The rate of flow is about 2 ml per minut.

The solution obtained by means of this flow through the ion exchanger is titrated coulometrically in the following manner: 5 ml of this solution is placed together with 20 ml of catholyte and 3 drops of the appropriate indicator into the cathode solution of the electrolytic vessel. We utilized a current intensity of 50 mA. At this current the rate of reaction is most suitable for noticing the color change of the indicator.

Because of the presence of sulfuric acid within the catholyte it is necessary to carry out a control reaction composed of 20 ml of catholyte and 3 drops of the corresponding indicator only. The control reaction should be coulometrically titrated to the same intensity of the indicator color as was obtained during the determination of the solution under analysis.

The quantity of the substance which is being determined follows from the Faraday laws. The number of Coulombs is obtained by subtracting the potassium sulfate, sodium acetate, sodium citrate, sodium nitrate, and sodium sulfate. By passing solutions of these salts through the cation exchanger "Merck" I, we produced corresponding acids which we titrated in the cathode

compartment utilizing appropriate indicators.

Results and Discussion

The results obtained show that this method can be applied, with satisfactory accuracy, to the determination of these salts.

(See Tables with English headings in original text)

(See Summary in English, p. 51)

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Tabela I — Table I

Kalijum-natrijum-tartarat — Potassium sodium tartrate

Indikator: fenoftalein
Indicator: phenophtalein
Supstanca sušena na 130°C do konstantne težine.
Substance dried to constant weight at 130°C.

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration: Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration: Nađena količina Quantity found mg
10	9,95	9,95
10	9,96	9,96
10	9,95	9,94
10	9,95	9,94
10	9,97	9,96
10	9,96	9,95
10	9,94	9,95

Tabela II — Table II

Kalijum nitrat — Potassium nitrate

Indikator: metil-crveno
Indicator: methyl-red

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration: Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration: Nađena količina Quantity found mg
10	9,96	9,95
10	9,98	9,96
10	9,98	9,96
10	9,97	9,95
10	9,99	9,98
10	9,97	9,96
10	9,99	9,98

Tabela III — Table III

Kalijum tartarat — Potassium tartrate

Indikator: fenoftalein
Indicator: phenolphtalein
Supstanca sušena na 155°C do konstantne težine
Substance dried to constant weight at 155°

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration: Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration: Nađena količina Quantity found mg
10	10,01	10,00
10	10,01	10,02
10	10,04	10,03
10	9,99	10,00
10	9,99	10,00
10	10,04	10,03
10	10,00	10,00

Tabela IV — Table IV

Kalijum sulfat — Potassium sulfate

Indikator: metil-crveno
Indikator: methyl-red

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration: Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration: Nađena količina Quantity found mg
10	9,99	9,97
10	9,98	9,97
10	9,97	9,96
10	9,98	9,97
10	9,98	9,98
10	9,97	9,97
10	9,99	9,98

Tabela V — Table V

Natrijum acetat — Sodium acetate

Indikator: fenolftalein
Indicator: phenolphtalein

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration: Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration: Nađena količina Quantity found mg
10	10,04	10,05
10	10,06	10,06
10	10,03	10,04
10	10,05	10,05
10	10,06	10,05
10	10,04	10,05
10	10,05	10,04

Tabela VI — Table VI

Natrijum citrat — Sodium citrate

Indikator: fenolftalein
Indicator: phenolphtalein
Supstanca sušena na 150°C do konstantne težine
Substance dried to constant weight at 150°C

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration: Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration: Nađena količina Quantity found mg
10	10,03	10,02
10	10,02	10,02
10	10,04	10,03
10	10,04	10,03
10	10,05	10,06
10	10,03	10,02
10	10,02	10,02

Tabela VII — Table VII

Natrijum nitrat — Sodium nitrate

Indikator: metil-crveno
Indicator: methyl-red

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration: Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration: Nađena količina Quantity found mg
10	9,98	9,99
10	9,98	9,99
10	9,98	9,98
10	9,99	10,00
10	9,98	9,99
10	9,99	10,00
10	9,97	9,98

Tabela VIII — Table VIII

Natrijum sulfat — Sodium sulfate

Indikator: metil-crveno
Indicator: methyl-red

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration: Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration: Nađena količina Quantity found mg
10	9,95	9,93
10	9,96	9,94
10	9,96	9,94
10	9,98	9,96
10	9,98	9,96
10	9,99	9,96
10	9,98	9,95

Coulometric determination of salts

K. Nikolić and R. Popović

SUMMARY

By the coulometric method a quick determination of salts is possible. Thus a new possibility for applying coulombs as a measuring unit is realized and the use of standard solution is avoided.

Appraritus. — As D. C. source a rectifier with a constant current in the range of 0—10 mA was used. The electrodes were made of platinum. To avoid undesirable changes in the anode compartment during electrolysis, the cathode and the anode compartments should be separated. The anode is put in a small Gooch pot with a layer of agar on its bottom. Then the pot is submerged into the liquid of the cathode compartment. The supporting electrolyte-catholyte-comprises 1 M sodium sulphate plus 10→ N sulphuric acid. The anolyte comprises 1 M sodium sulphate.

Procedure. — 100 mg of salt is to be taken and dissolved in 10 ml of water. This solution is then passed through a strong cation exchanger, »Merck« I. The column is to be rinsed with 5 ml until the volume of solution which passed through the column comes to 50 ml.

5 ml of this solution is then to be put into the cathode compartment and after that 20 ml of catholyte and this of an appropriate indicator are added to it. All this is then coulometrically determined. At the same time, taking 20 ml of catholyte and 3 drops of an appropriate indicator, the blank is determined.

The quantity of coulombs required for the titration of the analysed solution, diminished by the number of coulombe used for the blank determination, gives the quantity of salts.

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Završne tačke titracije odredili smo odgovarajućim indikatorima. Jugoslavenska farmakopeja II preporučuje za određivanje azotne i sumporne kiseline metil-oranž, koji nam nije odgovarao, jer je prelaz boje bio vrlo nejasan, pa su i rezultati određivanja nereproduktivni. Pošto farmakopeja USP i Internacionalna farmakopeja propisuju metil-crveno, upotrebili smo i mi ovaj indikator. On se pokazao veoma prikladnim pri svim određivanjima, jer je promena u boji mnogo jasnije izražena. Kako je rastvor natrijum-sulfata neutralan a promena boje ovog indikatora je u kiseloj sredini (4,40—6,20), morali smo da dodajemo 1 ml 0,1 N sumporne kiseline na 100 ml rastvora natrijum-sulfata koji je služio kao katolit. Zbog određivanja slepog opita pod istim uslovima, dodavanje kiseline nije uticalo na tačnost određivanja.

EKSPERIMENTALNI DEO

Reagensi i hemikalije:

a) Rastvor za katodni prostor (katolit): 142,05 g natrijum-sulfata rastvori se u vodi i vodom dopuni do 1000 ml, a zatim se doda 10 ml 0,1 N sumporne kiseline i dobro promeša.

b) Rastvor za anodni prostor (anolit): u vodi se rastvori 142,05 g natrijum-sulfata i vodom dopuni do 1000 ml.

Indikatori:

— Metil-crveno: 0,1 g se rastvori u 100 ml 70% etanola.

— Fenoftalein: 1 g rastvori se u 70 ml koncentrovanog etanola i rastvor razblaži vodom do 100 ml.

U postupku određivanja soli koristili smo i 0,01 N rastvor natrijum-hidroksida koji se može praviti kulometrijskim postupkom.

Za izmenu jona upotrebili smo smolu jako kiselog katijonskog izmenjivača »Merck« I.

Aparatura. — Za dobijanje jednosmerne stabilne struje upotrebljavali smo selenski ispravljač koji je preko transformatora i stabilizatora vezan za gradsku mrežu. Jačinu struje menjali smo preko promeljivog otpornika i transformatora sa kontinualnom promenom napona od 0 do 280 V. Elektrode su od platine. Katodni prostor odvajali smo preko jedne posude sa poroznim dnom. Da ne bi došlo do mešanja katolita sa anolitom na dno ove posude postavili smo agar-agar u kome je rastvoren kalijum-natrat.

Za merenje jačine struje koristili smo precizni miliampermetar »Iskra«.

Priprema stuba za izmenu jona.— Na dno staklene cevi prečnika 1 cm postavi se staklena vuna a zatim se cev napuni smolom izmenjivača. Zbog održavanja kompaktnosti stuba treba i na vrh stuba postaviti tampon staklene vune. Visina stuba treba da iznosi 12—14 cm. Pre punjenja stuba smola mora da bubri u vodi 24 sata.

Pre propuštanja rastvora mora se stub aktivirati tako da se kroz nj propušta 25 ml 10%-tnog rastvora hlorovodonične kiseline a zatim ispire destilovanom vodom do negativne reakcije na hloride.

Regenerisanje izmenjivača jona. — Posle svake upotrebe izmenjivač se regeneriše propuštanjem 25 ml 10%-tnog rastvora hlorovodonične kiseline. Po završenom propuštanju stub se ispire vodom do negativne reakcije na hloride.

Postupak. — Rastvor za kulometrijsku titraciju pripremili smo na ovaj način: Odmeri se 100 mg soli i rastvori u 10 ml svеže prokuvane

destilovane vode; rastvor se propušta kroz stub katijonskog izmenjivača koji je prethodno aktiviran. Čaša u kojoj je bio rastvor ispire se sa po 5 ml vode a zatim se ova propušta kroz stub. Stub se ispire vodom tako dugo dok zapremina rastvora, koji je prošao kroz stub, ne iznese 50 ml, a hvata se u odmerni balon od 50 ml, koji je postavljen ispod staklene cevi u koju je smešten izmenjivač jona. Brzina otkapavanja iznosi oko 2 ml na minut.

Rastvor dobijen propuštanjem kroz jonski izmenjivač, kulometrijski se titrira na ovaj način: u katodni se rastvor posude za elektrolizu stavi 5 ml ovog rastvora, 20 ml katolita i 3 kapi odgovarajućeg indikatora. Koristili smo jačinu struje od 50 mA. Pri ovoj jačini struje brzina reakcije je najpodesnija da bi se zapazila promena boje indikatora.

Zbog prisustva sumporne kiseline u katolitu potrebno je izvršiti i slepi opit tako da on sadrži samo 20 ml katolita i 3 kapi odgovarajućeg indikatora. Slepi opit treba kulometrijski titrirati do istog intenziteta boje indikatora dobijenog pri određivanju analiziranog rastvora.

Količinu supstancije koja se određuje izračunava se iz Faradejevih zakona. Broj kulona dobija se oduzimanjem broja utrošenih kulona za titraciju slepog opita od broja utrošenih kulona za titraciju analiziranog rastvora.

Uporedo sa kulometrijskim postupkom određivali smo i volumetrijski sa 0,01 N rastvorom natrijum-hidroksida.

Rezultati i diskusija

U tabelama navodimo rezultate dobijene kulometriskom i volumetriskom titracijom.

Tabela I — Table I

Kalijum-natrijum-tartarat — Potassium sodium tartrate

Indikator: fenoftalein
Indicator: phenophthalein
Supstanca sušena na 130°C do konstantne težine.
Substance dried to constant weight at 130°C.

	Kulometrijska titracija Coulometric titration	Volumetrijska titracija Volumetric titration
Uzeta količina Taken amounts mg	Nađena količina Quantity found mg	Nađena količina Quantity found mg
10	9,95	9,95
10	9,96	9,96
10	9,95	9,94
10	9,95	9,94
10	9,97	9,96
10	9,96	9,95
10	9,94	9,95

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»ACTA PHARMACEUTICA« JUGOSLAVICA« izlazi četiri puta na godinu. — Vlasnik i izdavač *Savez farmaceutskih društava Jugoslavije*, Beograd, M. Pijade 12. — Za uredništvo odgovara prof. *dr Ivan Stivić*, Zagreb, Ilica 104. — Pretplata za godinu 1966. (4 broja) iznosi za ustanove (apoteke, poduzeća, zavode i sl.) *n. D. 50.—*, za fizičke osobe *n. D. 10.—*; pretplata za inozemstvo iznosi 6 $. Pojedini broj stoji *n. D. 12.50*, a dvobroj *n. D. 25.—*. Pretplata se šalje na tekući račun kod Narodne banke, Zagreb, broj 3071-8-323. Časopis se tiska u Radionicama Grafičkog centra u Zagrebu, Trg maršala Tita br. 9.

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Sodium Citrate 268

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Kulometrijsko određivanje soli

Coulometric determination of salts

Kosta Nikolić i Radomir Popović

Predložena je kulometrijska metoda za određivanje kalijumovih i natrijumovih soli. Ove soli titrirali smo u katodnom prostoru posredstvom električne struje i količinu određivali iz broja utrošenih kulona.

A new method for determination of potassium and sodium salts was proposed. These salts were titrated in the cathode compartment by means of electrical current and judging by the number the coulombs used the quantity of salts was determined.

U Jugoslovenskoj farmakopeji II kao i u drugim farmakopejama postoje propisi za određivanje velikog broja kalijumovih i natrijumovih soli. Ovi su propisi zasnovani na klasičnim volumetrijskim i gravimetrijskim metodama. S obzirom na istaknuti značaj kulometrijske metode pokušali smo ovaj postupak primeniti na određivanje izvesnih kalijumovih i natrijumovih soli koje propisuje naša farmakopeja. Određivali smo ove soli: kalijum-natrijum-tartarat, kalijum-nitrat, kalijum-tartarat, kalijum-sulfat, natrijum-acetat, natrijum-citrat, natrijum nitrat i natrijum-sulfat. Ove su soli oficinalne, bilo kao materia medica ili pak kao reagencije.

Kako su to sve soli alkalnih metala, kulometrijskom smo određivanju mogli da pristupimo tek posle izvršene izmene jona. Koristeći jako kiseli kationski izmenjivač »Merck« I, izvršili smo proces izmene jona prikazan jednačinom:

$$\overset{-+}{R-SO_3H} + \overset{+-}{Me\,Y} \rightleftharpoons \overset{-+}{R-SO_3Me} + \overset{+-}{HY} \qquad (1)$$

Me — jon alkalnog metala

Pošto se pri ovoj izmeni oslobađa ekvivalentna količina jona vodonika, u eluatu smo dobili odgovarajuću kiselinu iz koje je postala so; ovu smo kiselinu mogli kulometrijski titrirati u katodnom prostoru. Iz broja utrošenih kulona mogli smo da izračunamo količinu soli koju smo određivali.

Sve soli što smo ih ispitivali dobro su se rastvarale u vodi, a takođe i kiseline koje smo dobili pri izmeni jona, pa smo kao rastvarač upotrebili svežo predestilisanu vodu.

Indikator: metil-crveno
Indicator: methyl-red

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration — Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration — Nađena količina Quantity found mg
10	9,96	9,95
10	9,98	9,96
10	9,98	9,96
10	9,97	9,95
10	9,99	9,98
10	9,97	9,96
10	9,99	9,98

Tabela III — Table III

Kalijum tartarat — Potassium tartrate

Indikator: fenolftalein
Indicator: phenolphtalein
Supstanca sušena na 155°C do konstantne težine
Substance dried to constant weight at 155°

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration — Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration — Nađena količina Quantity found mg
10	10,01	10,00
10	10,01	10,02
10	10,04	10,03
10	9,99	10,00
10	9,99	10,00
10	10,04	10,03
10	10,00	10,00

Tabela IV — Table IV

Kalijum sulfat — Potassium sulfate

Indikator: metil-crveno
Indikator: methyl-red

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration — Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration — Nađena količina Quantity found mg
10	9,99	9,97
10	9,98	9,97
10	9,97	9,96
10	9,98	9,97
10	9,98	9,98
10	9,97	9,97
10	9,99	9,98

Indikator: fenolftalein
Indicator: phenolphtalein

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration — Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration — Nađena količina Quantity found mg
10	10,01	10,05
10	10,06	10,06
10	10,03	10,04
10	10,05	10,05
10	10,06	10,05
10	10,01	10,05
10	10,05	10,04

Tabela VI — Table VI

Natrijum citrat — Sodium citrate

Indikator: fenolftalein
Indicator: phenolphtalein
Supstanca sušena na 150°C do konstantne težine
Substance dried to constant weight at 150°C

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration — Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration — Nađena količina Quantity found mg
10	10,03	10,02
10	10,02	10,02
10	10,04	10,03
10	10,04	10,03
10	10,05	10,06
10	10,03	10,02
10	10,02	10,02

Tabela VII — Table VII

Natrijum nitrat — Sodium nitrate

Indikator: metil-crveno
Indicator: methyl-red

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration — Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration — Nađena količina Quantity found mg
10	9,93	9,99
10	9,93	[illegible]
10	9,98	[illegible]
10	9,99	10,0[illegible]
10	9,98	[illegible]
10	9,99	10,[illegible]
10	9,97	9,[illegible]

Tabela VIII — Table VIII

Natrijum sulfat — Sodium sulfate

Indikator: metil-crveno
Indicator: methyl-red

Uzeta količina Taken amounts mg	Kulometrijska titracija Coulometric titration — Nađena količina Quantity found mg	Volumetrijska titracija Volumetric titration — Nađena količina Quantity found mg
10	9,95	9,93
10	9,98	9,94
10	9,96	9,94
10	9,98	9,96
10	9,98	9,96
10	9,99	9,96
10	9,98	9,95

Poredujući rezultate ova dva postupka možemo zaključiti da se elektrohemiske reakcije na elektrodama odvijaju željenim tokom. Pozitivna, odnosno negativna odstupanja od prvih vrednosti javljaju se kao posledica procesa izmene jona i nepoklapanja završne tačke titracije sa ekvivalentnom tačkom.

U Jugoslovenskoj farmakopeji II postoji samo propis za određivanje natrijum-citrata. Za većinu pomenutih soli farmakopeje Austrije, Čehoslovačke, Danske, Poljske, Rumunije i Sovjetskog Saveza daju metode određivanja i granice u kojima mora kretati sadržaj. Rezultati dobijeni našom metodom su unutar propisanih granica koje daju pomenute farmakopeje.

Zaključak

Kulometrijski smo određivali ove soli: kalijum-natrijum-tartarat, kalijum-nitrat, kalijum-tartarat, kalijum-sulfat, natrijum-acetat, natrijum-citrat, natrijum-nitrat i natrijum-sulfat. Propuštajući rastvore ovih soli kroz katijonski izmenjivač »Merck« I dobili smo odgovarajuće kiseline koje smo titrirali u katodnom prostoru upotrebljavajući odgovarajuće indikatore.

Dobijeni rezultati pokazuju da se ovaj postupak može sa zadovoljavajućom tačnošću primeniti za određivanje soli.

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Primljeno: 29. I 1968.

Coulometric determination of salts

K. Nikolić and R. Popović

SUMMARY

By the coulometric method a quick determination of salts is possible. Thus a new possibility for applying coulombs as a measuring unit is realized and the use of standard solution is avoided.

Appraritus. — As D. C. source a rectifier with a constant current in the range of 0—10 mA was used. The electrodes were made of platinum. To avoid undesirable changes in the anode compartment during electrolysis, the cathode and the anode compartments should be separated. The anode is put in a small Gooch pot with a layer of agar on its bottom. Then the pot is submerged into the liquid of the cathode compartment. The supporting electrolyte-catholyte-comprises 1 M sodium sulphate plus 10^{-3} N sulphuric acid. The anolyte comprises 1 M sodium sulphate.

Procedure. — 100 mg of salt is to be taken and dissolved in 10 ml of water. This solution is then passed through a strong cation exchanger, »Merck« I. The column is to be rinsed with 5 ml until the volume of solution which passed through the column comes to 50 ml.

5 ml of this solution is then to be put into the cathode compartment and after that 20 ml of catholyte and this of an appropriate indicator are added to it. All this is then coulometrically determined. At the same time, taking 20 ml of catholyte and 3 drops of an appropriate indicator, the blank is determined.

The quantity of coulombs required for the titration of the analysed solution, diminished by the number of coulombs used for the blank determination, gives the quantity of salts.

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Contribution to the pharmacology of piperazine

by Hans-Adolf Oelkers

Abstracts in German and English

In a summarizing representation "Activation methods of the electro-encaphalogram" R.S. Schwab (1) also mentioned the alkali charge of the organism by administering suitable alkali salts for several days. When daily doses of 2 to 5 g sodium citrate (2) were given 3 to 4 days in a row an increase of the attacks could be observed in patients with petit mal epilepsy. The same observation was made by Schuck and coworkers (3) after administering piperazine hexahydrate in the form of a syrup neutrailized with citric acid for several days to children suffering from oxyuriasis.

In previous investigations (4) it had been found in the animal test that the alkalinity of the urine is increased to about the same extent by equivalent doses of sodium nitrate and piperazine citrate. In toxicity determinations with subcutaneous injection, the median lethal dose of the two compounds was practically the same. The intoxication symptoms (muscle weakness, tremor, spasms) were also practically indentical.

According to animal tests by Mueller (5), sodium citrate is much more toxic in subcutaneous injection than sodium acetate, -lactate, -niocotinate and various other sodium salts of organic acids. In continuation of the tests with subcutaneous injection of sodium- and piperazine citrate, comparative toxicity determinations were made with oral application of these two salts and for comparison with piperazine tartrate. The much lower toxicity of the tartrate found in these tests suggested that the calcium-deionizing effect of the citric acid enhances the toxic effect of these two citrates.

For this reason tests were made on isolated guinea pig hearts and on isolated intestines of rabbits with different additions of these three salts to the nutrient solution. The object was to find out whether sodium citrate

and piperazine citrate show similar effects in corresponding concentrations under these conditions which differ from those of piperazine tartrate.

Schuch and coworkers (3) assumed on the basis of clinical observations an increase of the piperazine toxicity with simultaneous administration of anti-epileptics, but this assumption was later dropped as incorrect.

In view of the prostration syndrome described by Lorge (6), which can appear as a dangerous complication of the medicamentous treatment of epilepsy, even after doses had been well tolerated for a long time, for external reasons - temporary insufficient food intake, prolonged confinement to bed - and be misdiagnosed, we tested in the animal test whether the toxicity of phenobarbital and of diphenylhydantoin increases in the preliminary treatment with piperazine citrate.

Methodology

The toxicity was determined on white mice weighing from 25 to 30 g. The application of the neutral or weakly alkaline solutions (pH = 7.2) was made with the esophageal sound indicated by BEhrens in doses of 0.05 to 0.25 ml/10g. The 40% to 50% solutions were produced immediately before use under slight heating. The animals, which were kept in groups of 5 - five animals each received the same dose - were observed for 72 hours and the median lethal dose was calculated according to Kaerber.

Sodium citrate was used in all tests as natrium citricum neutrale dihydrat. p.a. (mol. weight 294.11) by Merck. 1 g of this salt is equivalent in alkalinity to 0.99 g piperazine hexahydrate.

The tests on the isolated guinea pig heart were carried out with the method by Langendorff, modified by Trunzler and Schuler (7).

For the tests on the isolated intestine of the rabbit (according to Magnus) we also used the tyrode solution described by Trunzler and Schuler. It contains 0.8% NaCl, 0.02% KCl, 0.012% CaCl2 (anhydrous), 0.01% MgCl2 + 6 H20, 0.1% NaHCO3, 0.005% NaH2PO4 + 2 H20 and 0.1% glucose.

In order to test the toxicity increase of phenobarbital and diphenylhydantoin (sodium-5,5-diphenyl-hydantoinate) by piperazine, we determined at first the median lethal doses of the two anti-epileptics (in 2% emulsion with stabilized gum arabic mucilage) first on white mice weighing 25 to 30 g with oral application. Subsequently the toxicity test was repeated on white mice which had received three days in a row twice daily and on the 4th day once 1.0 g/kg piperazine hexahydrate (as citrate) in the form of a 20% solution by means of the esophageal sound. About 1 to 1½ hours after the last piperazine dose the toxicity determinations were carried out with phenobarbital and diphenylhydantoin.

Results

The toxicity tests showed that the highest dose of sodium citrate that was still survived by all animals with oral application was 4.8 g/kg. But a short time after the application tremor and brief spasms appeared, and for about 24 hours great debility. Slightly higher doses were already lethal in some animals, but only the application of 9.6 g/kg mouse was not survived by any animal. Lethal doses lead to death after great convulsions usually within 45 to 60 minutes, rarely in the course of 2 to 3 hours. The median lethal dose was calculated with 7.12 g/kg.

The application of 5.6 g/kg piperazine hexahydrate (as citrate) was survived by all animals. Here too there were several convulsions during the first two hours and subsequently great debility. Only the application of 9.6 g/kg was lethal in all cases. The DL/50 calculated according to Kaerber was 7.6 g/kg and thus differed only slightly from that of sodium citrate.

Similar as in the tests with sodium citrate death occurred after lethal doses in the following 1 to 2 hours after violent convulsions. Only a small portion of the animals died after 4 to 6 hours, very rarely after 20 to 30 hours in a state of complete debility. Surviving mice recovered completely in the course of 2 to 3 days.

When piperazine hexahydrate was given in the form of tartrate, the dangerous doses started only at 7.0g/kg. The application of 12.0 g/kg was not survived by any animal. The median lethal dose was 9.2 g/kg, and was thus clearly higher than with the other two salts. Loss of toxicity and great debility of the animals, which hardly reacted to painful stimuli dominated the picture. Convulsions were absent or only minor (tremor, slight twitching). Breathing which was first increased and deeper, deteriorated in the further course. Death occurred after the highest doses only sporadically after 1 hour, in most cases 2 to 4 hours after the application, and, as in the test series with piperazine citrate, apparently from respiratory paralysis.

Just as in the toxicity determinations with sodium citrate and piperazine citrate, the pH-values measured in the urine with indicator paper rose clearly, as expected, after piperazine tartrate (from pH 6 to 6.4 to 6.8 to 7.7), since tartaric acid is burnt up relatively fast in the organism after resorption. Hyperpnea, which set in soon after the application of lethal and sublethal doses of the three salts, is probably another factor for the rising pH-values in the urine.

The tests on the isolated guinea pig heart showed that additions of equivalent doses of sodium- and piperazine citrate of the order of 20 mg% have about the same negatively inotropic effect. The heart beat decreased by about 10 to 15%. Fig. 1 and 2 show that the coronary perfusion clearly increased temporarily with the onset of the negatively inotropic effect. After we changed to pure tyrode solution, the animals soon recovered to the old state.

It can be assumed that ¼ to 1/3 of the calcium-ions found in the circulating liquid by the selected citrate additions are bound. This was shown in a test where the heart was temporarily traversed by a tyrode solution whose calcium-chloride content had been produced from 12.0 to 9.0 mg%. The effect was practically the same as in the tests with the citrate additions, and could be washed out relatively easily if the tests did not last too long. (fig.3).

Fig. 1 Isolated guinea pig heart. From to to bottom: contraction amplitude,

coronary perfusion (5 ml), time marking (1 min.) with 1 switching to tyrode solution with 20 mg% sodium citrate; with two switchings to pure tyrode solution.

Fig. 3 Isolated guinea pig heart. From to to bottom: contraction amplitude; coronary perfusion (5 ml); time marking (1 min.); with 1 switching to tyrode solution with reduced CaCl2-constant (9 mg%); with 2 switchings back to normal tyrode solution.

Fig. 4 Isolated guinea pig heart. From top to bottom: A contraction amplitude; coronary perfusion (5 ml); time marking; at arrow switching to tyrode solution with 20 mg% piperazine hexahydrate (neutralized with HCl).

Fig. 5 Isolated guinea pig heart. From top to bottom: contraction amplitude; coronary perfusion (5 ml); time marking (1 min); at arrow switching to tyrode solution with 20 mg% piperazine hexahydrate (neutralized with tartaric acid).

Fig. 6 Isolated rabbit intestine. At black circle addition of 30 mg piperazine hexahydrate as tartrate to 100 ml bath liquid; time marking = 1 minute.

Neutralized with hydrochloric acid, the addition of 20 mg% piperazine-hexahydrate to the tyrode solution had a slight positively inotropic effect on the heart, with a pronounced increase of the coronary perfusion (fig.4). If the same piperazine concentration was neutralized with tartaric acid, the positively inotropic effect was absent, and on the contrary a slight negatively inotropic effect was observed after a prolonged test (fig.5). The heart best increases during the perfusion with piperazine hydrochooride and piperazine tartrate by 5 to 10%.

Fig. 7 Isolated rabbit intestine. At black circle addition of 60 mg piperazine-hexahydrate as tartrate to 100 ml bath liquid. Time marking = 1 minute.

Fig. 8 Isolated rabbit intestine. At black circle addition of 30 mg piperazine-hexahydrate as citrate to 100 ml bath liquid. Time marking = 1 minute.

Fig. 9 Isolated rabbit intestine. At black circle addition of 60 mg sodium citrate to 100 ml bath liquid. Time marking = 1 minute.

Fig. 10 Isolated rabbit intestine. At black circle addition of 120 mg piperazine-hexahydrate as citrate to 100 ml bath liquid. Time marking = 1 min.

In the tests on the isolated intestine (jejunum), the addition of 30 mg piperazine hexahydrate as tartrate to 100 ml bath liquid caused a vigorous tonicity increase and a clear increase of the length of stroke. (fig.6). Both effects were more pronounced with double the piperazine tartrate concentration. (fig.7, but the tonicity increase usually lasted only a few minutes.

When piperazine hexahydrate was added in the same concentrations (30 and 60 mg%)as citrate to the nutrient solution, there was likewise a tonicity increase (fig.8), but the contraction movements slowed down considerably and were irregular. The same effect as with piperazine citrate was achieved with sodium-citrate (fig. 9).

Higher piperazine concentrations (from 100 to 120 mg%) led rapidly to a great drop of the tonicity of the intestine. The size of the contraction movements likewise decreased considerably. But while approximately normal conditions were restored in the course of 10 to 15 minutes if piperazine was added to the nutrient solution as tartrate, the addition of a corresponding amount of piperazine citrate did not have the same effect (fig. 10). The old value of the tonicity was not achieved again and the contraction movements were very weak, very slow and irregular. The equivalent amount of sodium citrate (100 mg%) always led within a few minutes to the complete disappearance of the motor field with a great drop of the tonicity. The effect could no longer be washed out at this stage.

The toxicity determinations with the oral application of phenobarbital on white mice weighing 25 to 30 g showed that the dangerous doses start at 140 g/kg. After a state of great excitation, which usually lasted about 1 hour, occasionally with brief convulsions there was restless sleep which

deepened to narcosis in the course of another hour. Death occurred rarely after 5 to 6 hours, even after positively lethal doses (250 mg/kg), usually during the following night, that is 12 to 20 hours after the application.

The surviving animals recovered very slowly in the following 2 to 3 days. Occasionally death occurred on the 2nd or 3rd day. The DL/50 was 215 mg/kg.

In the toxicity determinations with sodium diphenylhydantoinate the great excitation, interrupted by convulsions, was conspicuous, which developed 1½ to 2 hours after the application of higher doses (over 150 mg/kg) and which usually lasted for 1 to 2 days, diminishing gradually. Great numbness with reduced muscle tonus and devility alternated with phases of unrest and motor of varying duration.

Efforts to move were made difficult by imbalance, tremor, twitchings and fits of tonic-clonic spasms.

Death occurred after lethal doses - they started at 180 mg/kg, but only the double dose was lethal for all animals - generally only after 48 to 72 hours, occasionally only after 96 hours. The observation period was therefore 7 days. The DL/50 was 258 mg/kg.

In a repetition of the toxcity determinations on mice which had been treated with piperazine no difference was found from the result in normal animals of the same weight. The DL/50 of phenobarbital was in this test series 208 mg/kg and that of diphenylhydantoinate was 245 mg/kg. Under the selected test conditions there is thus no increase of the toxicity of phenobarbital and diphenylhydantoin.

Discussion of the results

It was already pointed out in 1956 by Moessner (8) on the basis of the literature of that time that the toxicity of piperazine depends in oral applications to a great extent on the form of preparation. But this was not always taken into consideration in toxicity tests and led naturally to contradictory results. Thus the indication that the application of 1.0 g/kg piperazine hydrate is already lethal for 20% of the mice (Boecker and Erhardt (9)) was

probably the result of lye poisoning, since piperazine hexahydrate is a highly basic reacting substance which can have a caustic effect on muscous membrane in a 10 to 20% solution.

Cross and coworkers (10) compared - likewise on mice - the oral toxicity of piperazine hexahydrate as a citrate and as an adipate. The authors arrived at the conclusion that piperazine hexahydrate as a citrate (LD/50 = 4.3 g/kg) is much more toxic than if given in the form of the adipate (LD/50 = 11.4 g/kg.)

This corresponds in principle to the above described comparative toxicity determinations with the citrate and tartrate of piperazine. Obviously the calcium-deionization by the citrate-ion is an essential factor which increases the toxicity. This is also indicated by the conspicuous agreement of the lethal doses of piperazine- and sodium citrate. The results of the tests on isolated guinea pig hearts and on the isolated intestine of the rabbit show clearly the practically identical effect of the two citrates under these circumstances.

It is no accident that the side effects in piperazine treatments described in the literature concern almost exclusively liquid preparations in which piperazine hexahydrate was present as a citrate. In this form piperazine seems to lead occasionally- with a given disposition- after several days corresponding to the above mentioned findings, to clinically detectable symptoms in addition to the eeg variations.

It seems that piperazine can cause in man too neurotoxic symptoms when taken accidentally in an overdose, most likely in the form of the citrate, while the adipate was tolerated without any sumptoms, even in extremely large single doses, according to Moessner (9). According to Kuelz (12), however, this piperazine salt led to the appearance of changes in the eeg, after prescribed doses have been taken regularly for several days, which were clearly found on the 7th day of the treatment in 80% of the children. The patients remained, however, clinically sumptom free.

Findings are also available from veterinarians which point in the same

direction. Thus Christoph and coworkers (11) saw in three of eight big cats, which had received a relatively high dose of piperazine citrate (300 mg/kg) in the treatment of worms, unrest, muscle twitching, ataxia, paralysis of the rear extremeties, mydriasis and salivation, followed by apathy and somnolence for about 24 hours. In 90 other young big cats, whose piperazine was administered in the same dose as hydrochloride or as adipate, no side effects were observed.

In the toxicity determinations with phenobarbital and diphenylhydantoin, no toxity increase of the two anti-spileptics was found under the selected test conditions. Nevertheless this possibility can not be excluded according to Schuch and coworkers (3), particularly since diphenylhydantoin can cause in large doses not only the prostation syndrome described by Lorge (6), which can be lethal under certain circumstances, but also convulsions lasting for several hours according to animal tests. But the lethal case of poisoning described by the authors - death occurred following a bronchoscopy by reflectory heart failure and respiratory standstill- can hardly be reconstructed in the animal test because, apart from diphenylhydantoin, Domnifen, Diamox, novacoin and pervitin were given among others repeatedly in high doses during the six days of treatment. Also difficult to evaluate is the importance of the circumscribed hemmorrhagic softening of the left parietal brain found in a dissection of the brain, which was a few days old.

References

(Translated by Carl Demrick Associates,Inc/IE/t)

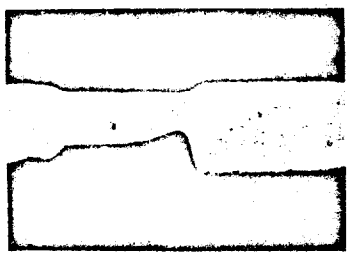
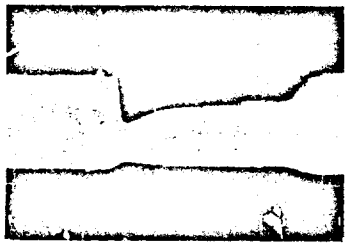


Figure 1

Figure 2



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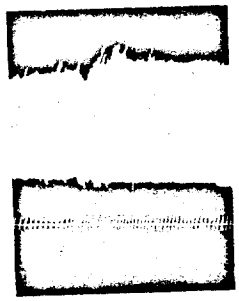

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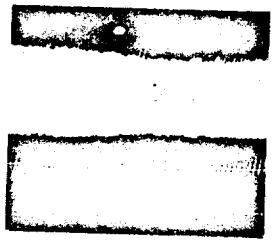

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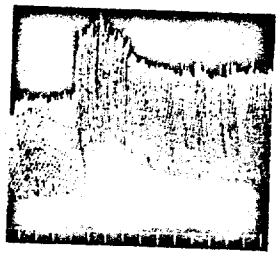

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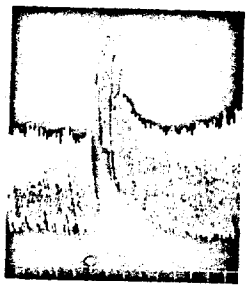
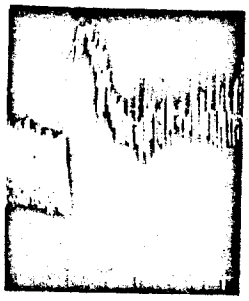


Figure 7

Figure 8



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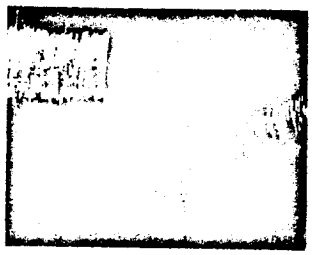

Figure 10

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HANS-ADOLF OELKERS

Beitrag zur Pharmakologie des Piperazins

Durch klinische Beobachtungen (*R. S. Schwab*) angeregte Tierversuche ergaben, daß die Toxizität von Piperazinzitrat bei oraler Verabfolgung sehr gering ist und derjenigen von Natriumzitrat weitgehend ähnelt. Die letale Dosis von Piperazintartrat lag bei den Versuchen deutlich höher als beim Piperazinzitrat und in toxischen Dosen konnte die nach Natrium- und Piperazinzitrat auftretende starke Krampfwirkung nicht beobachtet werden. Durch Versuche am isolierten Meerschweinchenherzen und am isolierten Kaninchendarm ließ sich zeigen, daß bei der Wirkung der beiden Zitrate die Kalziumentionisierung eine wesentliche Rolle spielt. Eine Giftigkeitssteigerung von Phenobarbital und Diphenylhydantoin konnte im Tierversuch durch mehrtägige Vorbehandlung mit Piperazinzitrat nicht festgestellt werden.

Tests on animals, inaugurated by clinical observations (*R. S. Schwab*) demonstrated, that the toxicity of piperazine citrate, orally given is low and quite similar to the toxicity of sodium citrate. The lethal dose of piperazine tartrate was significant higher in comparison to piperazine citrate. In toxic doses the strong convulsive effect, typical for sodium and piperazine citrate, could not be observed. Tests on isolated hearts of guinea-pigs and isolated intestine of rabbits demonstrated, that both citric salts have a reducing effect on calciumionisation in contrary to the tartrates beeing a deciding factor of different efficacy. An increase of toxicity of phenobarbital and diphenylhydantoin in consequence of a premedication with piperazine citrate could not be observed in animal tests.

In einer zusammenfassenden Darstellung „Aktivationsmethoden des Elektroenzephalogrammes" erwähnte *R. S. Schwab* (1) auch die Alkalibelastung des Organismus durch mehrtägige Verabfolgung geeigneter Alkalisalze. Wurden Tagesdosen von 2 bis 5 g Natriumzitrat (2) 3 bis 4 Tage hintereinander gegeben, so konnte bei Patienten mit Petit-Mal-Epilepsie auch eine Steigerung der Anfallshäufigkeit verzeichnet werden. Die gleiche Beobachtung machten *Schuch* und Mitarbeiter (3) nach mehrtägiger Verabfolgung von Piperazinhexahydrat in Form eines mit Zitronensäure neutralisierten Sirups bei der Behandlung der kindlichen Oxyuriasis.

Bei früheren Untersuchungen (4) war im Tierversuch gefunden worden, daß die Alkalität des Harns durch äquivalente Dosen von Natriumzitrat und Piperazinzitrat in etwa gleicher Weise zunimmt. Bei Toxizitätsbestimmungen mit subkutaner Injektion war die mittlere letale Dosis der beiden Verbindungen nahezu die gleiche. Auch die Vergiftungssymptome (Muskelschwäche, Zittern, Krämpfe) stimmten weitgehend überein.

Nach Tierversuchen von *Müller* (5) ist Natriumzitrat bei subkutaner Injektion erheblich giftiger als z. B. Natriumazetat, -laktat, -nikotinat und verschiedene andere Natriumsalze organischer Säuren. In Fortsetzung der Versuche mit subkutaner Injektion von Natrium- und Piperazinzitrat wurden nunmehr vergleichende Toxizitätsbestimmungen mit oraler Verabfolgung dieser beiden Salze sowie zum Vergleich mit Piperazintartrat vorgenommen. Die sich dabei herausstellende deutlich geringere Giftigkeit des Tartrats legte den Gedanken nahe, daß bei der toxischen Wirkung der beiden Zitrate der kalziumentionisierende Effekt der Zitronensäure eine unterstützende Rolle spielt.

Aus diesem Grund wurden Versuche am isolierten Meerschweinchenherzen und am isolierten Kaninchendarm mit verschiedenen Zusätzen dieser drei Salze zur Nährlösung durchgeführt. Hierbei sollte geprüft werden, ob Natriumzitrat und Piperazinzitrat in entsprechenden Konzentrationen auch unter diesen Bedingungen ähnliche Wirkungen haben, die sich von denen des Piperazintartrats unterscheiden.

Von *Schuch* und Mitarbeitern (3) wurde auf Grund klinischer Beobachtungen an eine Steigerung der Piperazintoxizität bei gleichzeitiger Verabfolgung von Antiepileptika gedacht, diese Annahme aber später als nicht zutreffend fallengelassen.

Im Hinblick auf das von *Lorgé* (6) beschriebene Prostationssyndrom, das als gefährliche Komplikation der medikamentösen Epilepsiebehandlung auch nach lange Zeit gut vertragenen Dosen aus äußeren Gründen — zeitweilig ungenügende Nahrungsaufnahme, Bettlägerigkeit — auftreten und verkannt werden kann, wurde daher im Tierversuch geprüft, ob die Toxizität von Phenobarbital und von Diphenylhydantoin bei Vorbehandlung mit Piperazinzitrat zunimmt.

Methodisches

Die Toxizitätsbestimmungen wurden an weißen Mäusen im Gewicht von 25 bis 30 g vorgenommen. Die Applikation der neutralen bzw. schwach alkalischen (pH 7,2) Lösungen erfolgte mit der von *Behrens* angegebenen Schlundsonde in Dosen von 0,05 bis 0,25 ml/10 g. Die 40 bis 50%igen Lösungen wurden unmittelbar vorher unter leichtem Erwärmen hergestellt. Die in Gruppen zu je 5 gehaltenen Tiere — je 5 Tiere erhielten die gleiche Dosis — wurden 72 Stunden beobachtet und die Berechnung der mittleren letalen Dosis nach *Kärber* vorgenommen.

Natriumzitrat wurde bei allen Versuchen als Natrium citricum neutrale dihydrat. p. a. (Mol. Gew. 294,11) von Merck gebraucht. Es ist 1,0 g dieses Salzes hinsichtlich Alkalität 0,99 g Piperazinhexahydrat äquivalent.

Die Versuche am isolierten Meerschweinchenherzen wurden mit dem von *Trunzler* und *Schuler* (7) modifizierten Verfahren von *Langendorff* durchgeführt.

Auch für die Versuche am isolierten Kaninchendünndarm (nach *Magnus*) fand die von *Trunzler* und *Schuler* angegebene Tyrodelösung Verwendung. Sie enthält 0,8% NaCl, 0,02% KCl, 0,012% $CaCl_2$ (wasserfrei), 0,01% $MgCl_2 + 6H_2O$, 0,1% $NaHCO_3$, 0,005% $NaH_2PO_4 + 2H_2O$ und 0,1% Glukose.

Zur Prüfung auf Giftigkeitssteigerung von Phenobarbital und Diphenylhydantoin (Natrium-5,5-Diphenyl-hydantoinat) durch Piperazin wurden die mittleren letalen Dosen der beiden Antiepileptika (in 2%iger Emulsion mit Mucilago Gummi arabici stabilisiert) zunächst an 25 bis 30 g schweren weißen Mäusen bei oraler Applikation bestimmt. Anschließend wurde die Toxizitätsprüfung an weißen Mäusen wiederholt, die drei Tage hintereinander zweimal am Tag und am 4. Tag einmal 1,0 g/kg Piperazinhexahydrat (als Zitrat) in Form einer 20%igen Lösung mittels Schlundsonde erhalten hatten. Ca. 1 bis 1½ Std. nach der letzten Piperazindosis wurden die Toxizitätsbestimmungen mit Phenobarbital und Diphenylhydantoin durchgeführt.

Ergebnisse

Die Toxizitätsprüfungen zeigten, daß vom Natrium citricum die höchste Dosis, die bei oraler Verabfolgung noch von allen Tieren überlebt wurde, 4,8 g/kg betrug. Dabei traten aber kurze Zeit nach der Applikation Zittern, kurzdauernde Krampfanfälle und für ca. 24 Stunden starke Hinfälligkeit ein. Bereits wenig höhere Dosen wirkten bei einigen Tieren tödlich, aber erst die Eingabe von 9,6 g pro kg Maus wurde in keinem Fall mehr überlebt. Letale Dosen führten nach heftigen Krämpfen gewöhnlich bereits innerhalb von 45 bis 60 min, selten erst im Verlauf der folgenden 2 bis 3 Stunden zum Tode. Die mittlere letale Dosis wurde mit 7,12 g/kg errechnet.

Von Piperazinhexahydrat (als Zitrat) wurde die Eingabe von 5,6 g/kg noch von allen Tieren überlebt. Auch hier kam es während der ersten beiden Stunden zu mehreren Krampfanfällen und anschließend zu starker Hinfälligkeit. Erst die Eingabe von 9,6 g/kg wirkte in allen Fällen tödlich. Die nach *Kärber* errechnete DL_{50} betrug 7,6 g/kg und unterschied sich mithin nur unerheblich von derjenigen des Natriumzitrats.

Ähnlich wie bei den Versuchen mit Natriumzitrat trat der Tod nach letalen Dosen nach mehreren heftigen Krampfanfällen häufig bereits im Verlauf der folgenden 1 bis 2 Stunden ein. Nur ein kleiner Teil der Tiere kam erst nach 4 bis 6 Stunden, sehr selten erst nach 20 bis 30 Stunden in einem Stadium äußerster Hinfälligkeit zum Exitus. Überlebende Mäuse erholten sich im Verlauf von 2 bis 3 Tagen vollständig.

Wurde Piperazinhexahydrat als Tartrat verabfolgt, so begannen die gefährlichen Dosen erst bei 7,0 g/kg. Die Eingabe von 12,0 g/kg wurde von keinem Tier überlebt. Die mittlere letale Dosis betrug 9,2 g/kg und lag mithin deutlich höher als bei den beiden anderen Salzen. Tonusverlust und starke Hinfälligkeit der auch auf schmerzhafte Reize kaum reagierenden Tiere beherrschten das Vergiftungsbild. Krämpfe fehlten oder waren nur angedeutet vorhanden (Zittern, leichte Zuckungen). Die zu Beginn vermehrte und vertiefte Atmung verschlechterte sich im weiteren Verlauf. Der Tod trat auch nach den höchsten Dosen nur vereinzelt bereits nach 1 Stunde, bei der Mehrzahl der Tiere erst 2 bis 4 Stunden nach der Eingabe und wie bei der Versuchsreihe mit zitronensaurem Piperazin, anscheinend an Atemlähmung ein.

Ebenso wie bei den Toxizitätsbestimmungen mit Natriumzitrat und Piperazinzitrat stiegen die mit Indikatorpapier gemessenen pH-Werte im Harn auch nach Piperazintartrat erwartungsgemäß deutlich an (von pH 6 bis 6,4 auf 6,8 bis 7,7), da auch Weinsäure nach der Resorption im Organismus relativ rasch verbrannt wird. Die Hyperpnoe, die bald nach der Eingabe subletaler und letaler Dosen der drei Salze einsetzte, dürfte ein weiterer Faktor für die steigenden pH-Werte im Harn sein.

Die Versuche am isolierten Meerschweinchenherzen ergaben, daß Zusätze äquivalenter Dosen von Natrium- und Piperazinzitrat in der Größenordnung von 20 mg% in ungefähr gleicher Stärke negativ inotrop wirken. Dabei nahm die Herzfrequenz um ca. 10 bis 15% ab. Die Abbildungen 1 und 2 zeigen, daß mit dem Einsetzen der negativ inotropen Wirkung der Koronardurchfluß vorübergehend deutlich zunahm. Nach dem Zurückschalten auf reine Tyrodelösung trat rasche Wiederherstellung des alten Zustandes ein.

Es ist anzunehmen, daß durch die gewählten Zitratzusätze ¼ bis ⅓ der in der Durchströmflüssigkeit vorhandenen Kalziumionen gebunden werden. Dies zeigte ein Versuch, bei dem das Herz vorübergehend mit einer Tyrodelösung durchströmt wurde, deren Gehalt an Kalziumchlorid von 12,0 auf 9,0 mg% herabgesetzt worden war. Der Effekt war praktisch der gleiche wie bei den Versuchen mit den Zitratzusätzen und bei nicht zu langer Versuchsdauer relativ leicht auswaschbar (Abbildung 3).

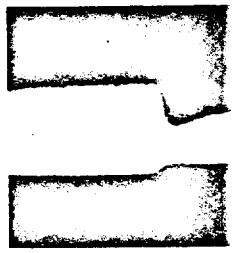
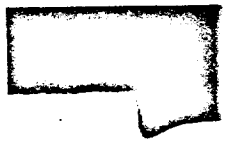


Abbildung
Isoliertes Meerschweinchenherz. Von
traktionsamplitude, Koronardurchfl
(1 min). Bei 1 Umschalten auf Ty
Piperazinhexahydrat (neutralisiert
2 Zurückschalten auf reine



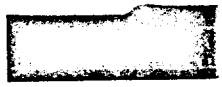


Abbildung
Isoliertes Meerschweinchenherz. Vo
traktionsamplitude, Koronardurchfl.
(1 min). Bei 1 Umschalten auf Ty
Natriumzitrat. Bei 2 Zurückschalten

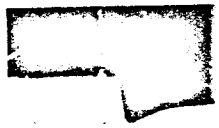


Abbildung
Isoliertes Meerschweinchenherz. V
traktionsamplitude, Koronardurchf
(1 min). Bei 1 Umschalten auf Ty
tem $CaCl_2$-Gehalt (9 mg%). Bei 2
Tyrodelösu

Mit Salzsäure neutralisie
von 20 mg% Piperazinhex
lösung eine leichte positiv i
das Herz, wobei der Koro
zunahm (Abbildung 4). Lag
konzentration mit Weinsäur

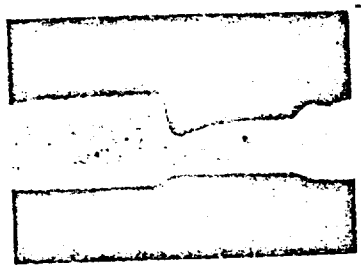


Abbildung 1

Isoliertes Meerschweinchenherz. Von oben nach unten: Kontraktionsamplitude, Koronardurchfluß (5 ml), Zeitmarkierung (1 min). Bei 1 Umschalten auf Tyrodelösung mit 20 mg% Piperazinhexahydrat (neutralisiert mit Zitronensäure). Bei 2 Zurückschalten auf reine Tyrodelösung.

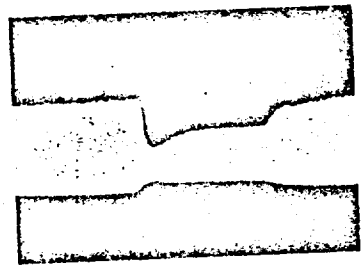


Abbildung 2

Isoliertes Meerschweinchenherz. Von oben nach unten: Kontraktionsamplitude, Koronardurchfluß (5 ml), Zeitmarkierung (1 min). Bei 1 Umschalten auf Tyrodelösung mit 20 mg% Natriumzitrat. Bei 2 Zurückschalten auf reine Tyrodelösung.

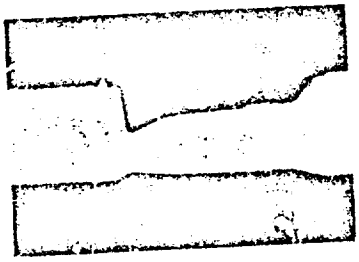


Abbildung 3

Isoliertes Meerschweinchenherz. Von oben nach unten: Kontraktionsamplitude, Koronardurchfluß (5 ml), Zeitmarkierung (1 min). Bei 1 Umschalten auf Tyrodelösung mit vermindertem $CaCl_2$-Gehalt (9 mg%). Bei 2 Zurückschalten auf normale Tyrodelösung.

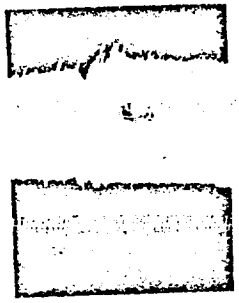


Abbildung 4

Isoliertes Meerschweinchenherz. Von oben nach unten: Kontraktionsamplitude, Koronardurchfluß (5 ml), Zeitmarkierung. Bei ↑ Umschalten auf Tyrodelösung mit 20 mg% Piperazinhexahydrat (mit HCl neutralisiert).

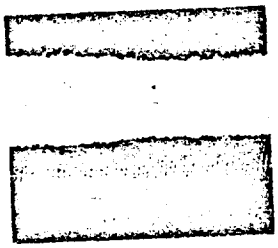


Abbildung 5

Isoliertes Meerschweinchenherz. Von oben nach unten: Kontraktionsamplitude, Koronardurchfluß (5 ml), Zeitmarkierung (1 min). Bei ↑ Umschalten auf Tyrodelösung mit 20 mg% Piperazinhexahydrat (mit Weinsäure neutralisiert).

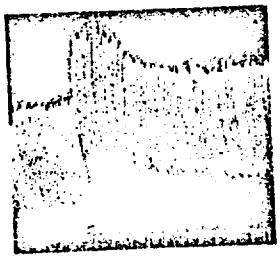


Abbildung 6

Isolierter Kaninchendarm. Bei ⊕ Zusatz von 30 mg Piperazinhexahydrat als Tartrat auf 100 ml Badflüssigkeit. Zeitmarkierung = 1 Minute.

Mit Salzsäure neutralisiert hatte der Zusatz von 20 mg% Piperazinhexahydrat zur Tyrodelösung eine leichte positiv inotrope Wirkung auf das Herz, wobei der Koronardurchfluß deutlich zunahm (Abbildung 4). Lag die gleiche Piperazinkonzentration mit Weinsäure neutralisiert vor, so fehlte der positiv inotrope Effekt, und es war im Gegenteil bei längerer Versuchsdauer eine geringe negativ inotrope Wirkung festzustellen (Abbildung 5). Die Herzfrequenz nahm während der Durchströmung mit salzsaurem und weinsaurem Piperazin um 5 bis 10% zu.

Abbildung 7
Isolierter Kaninchendarm. Bei ⊙ Zusatz von 60 mg Piperazinhexahydrat als Tartrat auf 100 ml Badflüssigkeit. Zeitmarkierung = 1 Minute.

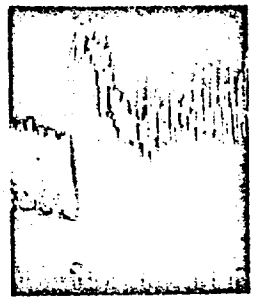


Abbildung 9
Isolierter Kaninchendarm. Bei ⊙ Zusatz von 60 mg Natriumzitrat zu 100 ml Badflüssigkeit. Zeitmarkierung = 1 Minute.

Abbildung 8
Isolierter Kaninchendarm. Bei ⊙ Zusatz von 30 mg Piperazinhexahydrat als Zitrat auf 100 ml Badflüssigkeit. Zeitmarkierung = 1 Minute.

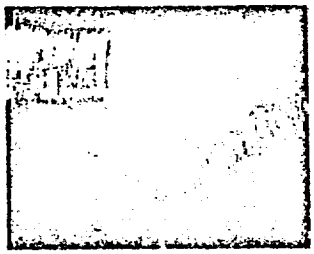


Abbildung 10
Isolierter Kaninchendarm. Bei ⊙ Zusatz von 120 mg Piperazinhexahydrat als Zitrat zu 100 ml Badflüssigkeit. Zeitmarkierung = 1 Minute.

Bei den Versuchen am isolierten Kaninchendarm (Jejunum) rief der Zusatz von 30 mg Piperazinhexahydrat als Tartrat zu 100 ml Badflüssigkeit kräftigen Tonusanstieg und deutliche Zunahme der Hubhöhe hervor (Abbildung 6). Beide Wirkungen waren bei der doppelten Piperazintartratkonzentration stärker (Abbildung 7), doch hielt der Tonusanstieg gewöhnlich nur wenige Minuten an.

Wurde Piperazinhexahydrat in den gleichen Konzentrationen (30 und 60 mg%) als Zitrat zur Nährlösung zugegeben, so kam es ebenfalls zur Tonuszunahme (Abbildung 8), wobei aber die Kontraktionsbewegungen gleichzeitig erheblich langsamer und ungleichmäßig erfolgten. Den gleichen Effekt wie Piperazinzitrat hatte auch Natriumzitrat (Abbildung 9).

Höhere Piperazinkonzentrationen (ab 100 bis 120 mg%) führten rasch zum starken Sinken des Darmtonus. Dabei nahm die Höhe der Kontraktionsbewegungen ebenfalls stark ab. Während sich aber im Verlauf von 10 bis 15 min annähernd normale Verhältnisse wiederherstellten, wenn Piperazin der Nährlösung als Tartrat zugesetzt wurde, war dies bei Zugabe der entsprechenden Piperazinzitratmenge nicht der Fall (Abbildung 10). Die alte Höhe des Tonus wurde nicht wieder erreicht und die Kontraktionsbewegungen erfolgten nur noch schwach, stark verlangsamt und ungleichmäßig. Die äquivalente Natriumzitratmenge (100 mg%) führte regelmäßig unter starkem Tonusabfall innerhalb weniger Minuten zum völligen Verschwinden der Motorik. Die Wirkung war in diesem Stadium nicht mehr auswaschbar.

Die Toxizitätsbestimmungen mit oraler Verabfolgung von Phenobarbital an 25 bis 35 g schweren weißen Mäusen ergaben, daß die gefährlichen Dosen bei 140 mg/kg beginnen. Stets stellte sich nach einem durchschnittlich 1 Stunde anhaltenden starken Erregungszustand — gelegentlich mit kurzdauernden Krampfanfällen — ein unruhiger Schlafzustand ein, der sich im Verlauf einer weiteren Stunde zur Narkose vertiefte. Der Tod trat auch nach sicher letal wirkenden Gaben (250 mg/kg) selten bereits nach 5 bis 6 Stunden, gewöhnlich aber erst während der folgenden Nacht, d. h. also 12 bis 20 Stunden nach der Eingabe ein.

Die überlebenden Tiere erholten sich nur langsam im Verlauf von 2 bis 3 Tagen. Vereinzelt traten Todesfälle noch am 2. oder 3. Tag ein. Als DL_{50} ergab sich 215 mg/kg.

Bei den Toxizitätsbestimmungen mit Natrium-Diphenylhydantoinat fiel die starke, von Krämpfen unterbrochene Erregung auf, die sich 1½ bis 2 Stunden nach der Eingabe höherer Dosen (> 150 mg/kg) entwickelte und die gewöhnlich 1 bis 2 Tage allmählich schwächer werdend anhielt. Starke Benommenheit mit herabgesetztem Muskeltonus und Hinfälligkeit wechselten mit kürzeren oder längeren Phasen von Unruhe und Bewegungsdrang ab.

Die Bemühungen, sich fortzubewegen, wurden durch Gleichgewichtsstörungen, Zittern, Zuckungen und Anfälle von tonisch-klonischen Krämpfen erschwert.

Der Tod trat nach letalen Dosen — sie begannen bei 180 mg/kg, für alle Tiere letal wirkte aber erst die doppelte Dosis — im allgemeinen erst nach 48 bis 72 Stunden, gelegentlich sogar erst nach 96 Stunden ein. Die Beobachtungsdauer betrug daher 7 Tage. Als DL_{50} wurde 258 mg/kg gefunden.

Bei der Wiederholung der Toxizitätsbestimmungen an mit Piperazin vorbehandelten Mäusen konnte kein Unterschied zu dem Ergebnis bei den gleich schweren Normaltieren festgestellt werden. Die DL_{50} von Phenobarbital betrug bei dieser Versuchsreihe 208 mg/kg und von Diphenylhydantoinat 245 mg/kg. Unter den gewählten Versuchsbedingungen tritt mithin eine Zunahme der Giftigkeit von Phenobarbital und Diphenylhydantoin nicht in Erscheinung.

Besprechung der Ergebnisse

Bereits 1956 wurde von *Mössner* (8) auf Grund der damals vorliegenden Literatur darauf hingewiesen, daß die Toxizität des Piperazins bei oraler Verabfolgung weitgehend von der Darreichungsform abhängig ist. Das wurde allerdings bei Toxizitätsprüfungen nicht immer berücksichtigt und führte naturgemäß zu widerspruchsvollen Ergebnissen. So dürfte sich die Angabe, wonach bei Mäusen die Eingabe von 1,0 g/kg Piperazinhydrat bereits für 20% der Tiere tödlich ist (*Boecker* und *Erhardt* [9]), als Folge einer Laugenvergiftung erklären. Handelt es sich doch beim Piperazinhexahydrat um eine stark basisch reagierende Substanz, die in 10 bis 20%iger Lösung auf Schleimhäute ätzend zu wirken vermag.

Cross und Mitarbeiter (10) verglichen — ebenfalls an Mäusen — die orale Toxizität von Piperazinhexahydrat als zitronensaures und als adipinsaures Salz. Die Autoren kamen zu dem Ergebnis, daß Piperazinhexahydrat als Zitrat (DL_{50} = 4,3 g/kg) erheblich giftiger ist als bei Eingabe in Form des Adipats (DL_{50} = 11,4 g/kg).

Dies entspricht im Prinzip den oben geschilderten vergleichenden Toxizitätsbestimmungen mit dem Zitrat und dem Tartrat des Piperazins. Offensichtlich ist die Kalziumentionisierung durch das Zitratanion ein wesentlicher Faktor, der die Giftigkeit erhöht. Hierfür spricht auch die auffällige Übereinstimmung der letalen Dosen von Piperazin- und Natriumzitrat. Die Ergebnisse der Versuche am isolierten Meerschweinchenherzen und am isolierten Kaninchendarm zeigen die unter diesen Umständen praktisch identische Wirkung der beiden Zitrate besonders deutlich.

Es dürfte kein Zufall sein, daß es sich bei den in der Literatur beschriebenen Fällen von Nebenwirkungen bei Piperazinkuren fast ausnahmslos um flüssige Präparate gehandelt hat, in denen Piperazinhexahydrat als Zitrat vorlag. In dieser Form scheint Piperazin bei mehrtägiger Verabfolgung, den eingangs erwähnten Befunden von *Schwab* entsprechend, außer zu EEG-Veränderungen bei vorliegender Disposition gelegentlich auch zu klinisch feststellbaren Erscheinungen zu führen.

Es scheint, daß Piperazin auch beim Menschen bei akzidenteller Einnahme einer Überdosis am ehesten als Zitrat neurotoxische Erscheinungen auszulösen vermag, wohingegen das Adipat nach *Mössmer* (9) in extrem hohen Einzeldosen symptomlos vertragen wurde. Nach *Külz* (12) führt aber auch dieses Piperazinsalz bei mehrtägiger regelmäßiger Einnahme der vorschriftsmäßigen Dosen zum Auftreten von Veränderungen im EEG, die am 7. Behandlungstag bei rund 80% der Kinder deutlich festzustellen waren. Klinisch blieben die Patienten allerdings unauffällig.

Von veterinärmedizinischer Seite liegen ebenfalls Befunde vor, die in die gleiche Richtung deuten. So sahen *Christoph* und Mitarbeiter (11) bei drei von acht jungen Großkatzen, die zur Entwurmung eine relativ hohe Dosis (300 mg/kg) Piperazinzitrat erhalten hatten, Unruhe, Muskelzuckungen, Ataxie, Lähmungserscheinungen an den hinteren Extremitäten, Mydriasis und Speichelfluß sowie anschließend Apathie und Somnolenz für etwa 24 Stunden. Bei 90 weiteren jungen Großkatzen, denen Piperazin in gleicher Dosierung als Hydrochlorid oder als Adipat eingegeben worden war, traten dagegen keinerlei Nebenwirkungen auf.

Bei den Toxizitätsbestimmungen mit Phenobarbital und Diphenylhydantoin war unter den gewählten Versuchsbedingungen keine Giftigkeitssteigerung der beiden Antiepileptika festzustellen. Trotzdem ist aber nach den Angaben von *Schuch* und Mitarbeitern (3) an diese Möglichkeit zu denken, zumal Diphenylhydantoin in hohen Dosen nicht nur das von *Lorgé* (6) geschilderte,

unter Umständen tödlich endende Prostrationssyndrom, sondern nach den Tierversuchen auch schwere, viele Stunden anhaltende Krämpfe hervorrufen kann. Allerdings läßt sich der von den Autoren geschilderte tödlich endende Vergiftungsfall — der Tod erfolgte im Anschluß an eine Bronchoskopie durch reflektorischen Herz- und Atemstillstand — im Tierversuch kaum rekonstruieren, da während des sechstägigen Verlaufes außer Diphenylhydantoin u. a. auch Somnifen, Diamox, Novocain und Pervitin sehr hoch dosiert und wiederholt gegeben wurden. Schwer zu beurteilen ist ferner die Bedeutung der bei der Sektion des Gehirns gefundenen wenige Tage alten, umschriebenen hämorrhagischen Erweichung im linken Parietalhirn.

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WALTER LÜHRS, ANNELIESE BESCH und ARNOLD REINCKE

Therapeutische Kombinationsversuche mit einem Chloroquinderivat und Trenimon bei Mäusen mit einem Ehrlich-Aszites-Tumor

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An Ehrlich-Aszites-Tumorzellen tragenden weißen Mäusen wurde in der Kombination mit Resochin und 50% einer therapeutisch wirksamen Trenimondosis eine annähernd gleiche Überlebenszeit erreicht, wie mit einer 100%igen Trenimon-Medikation allein. Interessant war die Versuchsanordnung, wobei sich der Ehrlich-Aszites-Tumor acht Tage lang in Ruhe ohne jegliche Beeinflussung entwickeln konnte und ab 9. Tag eine kombinierte Behandlung von Resochin und Trenimon in der 50%igen Dosis erfuhr. Hierbei konnte eine mäßige Verlängerung der Überlebenszeit beobachtet werden. Interessant war der praktisch parallele Verlauf der Überlebenszeit gegenüber primären therapeutischen Versuchsanordnungen.

In white mice with Ehrlich ascites tumor cells about the same survival rate was obtained with a combination of Resochin with 50 per cent of a therapeutical efficacious Trenimon dosis as with an exclusive Trenimon dosis. There was an interesting experimental setup: The Ehrlich ascites tumor was allowed to develop without any hindrance for eight days, whereupon on the ninth the treatment with the Resochin-Trenimon combination was begun. A moderate extension of the survival rate could then be observed. The almost parallel course of the survival time against primary therapeutical experimental setups was quite interesting.

Hiraki und Mitarbeiter fanden, daß Chloroquinderivate in der Gewebekultur das Wachstum von Fibroblasten beeinflussen konnten. In vivo wurden bei Ratten Granulome in ihrem Wachstum behindert. Tierversuche derselben Autoren zeigten nur eine deutliche Beeinflussung der Tumorarten, die reich an Bindegewebe waren. Bei *Ehrlich-* und *Yoshida*-Aszites-Tumoren wurde keine deutliche Wachstumshemmung festgestellt. Diese experimentellen Daten bewogen dieselben Autoren, Chloroquinderivate bei Menschen mit den verschiedensten Malignomen anzuwenden. In einer späteren Veröffentlichung (3) berichteten sie über 54 Fälle, wobei diese Substanz bei 38 Fällen effektvoll befunden wurde. 15 Fälle ließen keinerlei Beeinflussung erkennen. Die deutlichste

Beeinflussung wurde bei [illegible] Bronchial- oder einem Blasen-[illegible] einer weiteren Veröffentlichung [illegible] Autoren 11 Fälle von [illegible] handelt. Hierbei zeigten [illegible] Regressionen. Der Umfang der [illegible] konfluierenden Lymphome [illegible] sarkom zeigte wohl eine [illegible] Grund der klinischen Erfahrung [illegible] daß sie geringer war, als [illegible] ten Zytostatika. Ein gemischter [illegible] bei der Lymphogranulomatose [illegible] lumzell-Sarkom.

Diese Tatsachen bewogen [illegible] Mäusen mit einem Ehrlich-[illegible] Kombinationsversuche zu [illegible] Konzeption war, Kombinationen [illegible] bei das mit toxischen Nebenwirkungen [illegible] Zytostatikum in seiner Dosierung [illegible] werden könnte (5). Dazu [illegible] „unbekannter" Abwehrmechanismus [illegible] generalisierter Krebsformen [illegible] beträchtlichen toxischen Nebenwirkungen [illegible] *Schmähl* in Tierversuchen [illegible] (6).

Auf Grund dieser Überlegungen [illegible] folgende Tierversuche [illegible] kamen das Chloroquinderivat [illegible] Zytostatikum Trenimon [illegible] nen Inzuchtstamm CF: [illegible] folgenden Tierversuchen [illegible]

Folgende Versuchsanordnung [illegible]

1. Serie: 10 weiße Mäuse [illegible] lich-Aszites-Tumorzellen [illegible] Behandlung belassen.
2. Serie: 10 weiße Mäuse [illegible] lich-Aszites-Tumor bekämpft [illegible] Beginn mit einer täglichen [illegible] pergewicht = 0,05 mg Resochin.
3. Serie: 10 weiße Mäuse [illegible] Ehrlich-Aszites-Tumor bekämpft [illegible] lung mit 0,004 mg Trenimon.
4. Serie: 10 weiße Mäuse [illegible] lich-Aszites-Tumor bekämpft [illegible] mit 0,025 mg Resochin und [illegible]
5. Serie: 10 weiße Mäuse [illegible] lich-Aszites-Tumor bekämpft [illegible] rend der nächsten 8 Tage [illegible] 9. Tag Durchführung der Kombination wie unter 4.

1. *Hiraki, K.,* und *K.* [illegible] malignant Tumors [illegible] Fibroblast-inhibiting [illegible] Okayama. Vol. [illegible]
2. *Hiraki, K.,* und *K.* [illegible] on animal Tumors. [illegible]
3. *Hiraki, K.,* und *K.* [illegible] on Human Cancers [illegible] (1964).

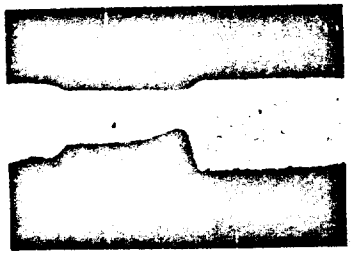

Figure 1

Figure 2

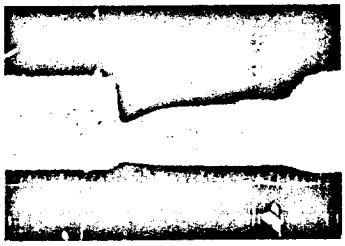

Figure 3

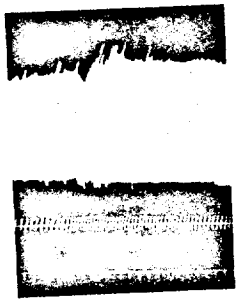

Figure 4

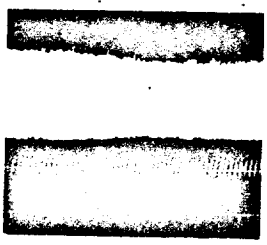

Figure 5

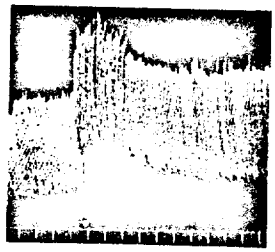

Figure 6

Figure 7

Figure 8

Figure 9

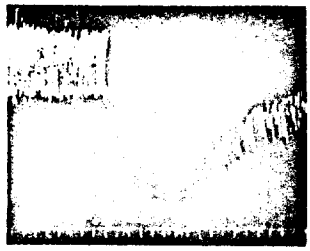

Figure 10

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THE RELATIVE TOXICITY IN RATS OF DISODIUM ETHYLENE DIAMINE TETRA-ACETATE, SODIUM OXALATE AND SODIUM CITRATE

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In previous work in this laboratory solutions of sodium oxalate or of disodium ethylene diamine tetra-acetate (disodium EDTA) were injected intravenously to induce experimental hypocalcaemia in cows (Payne, Sansom & Manston, 1963) and rats (Payne & Sansom, 1963). It appeared from experiments with cows that disodium EDTA induced hypocalcaemia more rapidly than sodium oxalate administered at an equivalent rate. Thus, given in sufficient quantity both agents induced symptoms, but with disodium EDTA these occurred when the plasma calcium concentration was at its lowest point, whereas with sodium oxalate they occurred when the plasma calcium concentration was returning to normal after the injection had ceased. The purpose of the present experiments was to investigate these differences. It was hoped that a study of the relative toxicity of disodium EDTA and sodium oxalate when administered intravenously to rats might reveal the nature of the mechanisms involved in the removal of calcium from the plasma. Sodium citrate was included in the investigation as another chelating agent of potential value for inducing hypocalcaemia.

METHODS

The rats used in these experiments were inbred hooded Norwegian or Wistar albino. All were in a good nutritional state.

Preparation of disodium EDTA, sodium oxalate and sodium citrate solutions. Disodium EDTA solution was prepared as previously described (Payne & Sansom, 1963), the stock solution being equivalent to 5·93 mg Ca/ml. Stock solutions of sodium oxalate and sodium citrate of theoretically equivalent calcium-precipitating or chelating power were also prepared. Where necessary dilutions were made with NaCl solution 9 g/100 ml.

Preparation of sodium oxalate containing freshly precipitated calcium oxalate and calcium phosphate. A precipitate of calcium oxalate was obtained by adding an excess of calcium chloride to 10 ml. of the stock solution of sodium oxalate. This was centrifuged and washed once with distilled water and once with stock sodium oxalate solution. It was then resuspended in either 100 ml. or 1 l. of stock sodium oxalate solution. These fluids therefore contained sodium oxalate equivalent to 5·93 mg Ca/ml. with the addition of calcium as calcium oxalate 0·593 mg or 0·0593 mg/ml., most of which was in suspension. For the

purpose of this paper they will be called sodium oxalate (CaOX 0·1) and sodium oxalate (CaOX 0·01) respectively.

Suspensions of calcium phosphate in sodium oxalate solutions were prepared similarly. Calcium phosphate was precipitated from 10 ml. of a solution of calcium chloride containing 5·93 mg calcium/ml. with excess sodium orthophosphate. The precipitate was washed and resuspended in either 100 ml. or 1 l. of stock sodium oxalate solution. These solutions, which contained calcium as calcium phosphate either 0·593 mg or 0·0593 mg/ml., will be called sodium oxalate ($Ca_3(Po_4)_2$ 0·1) and sodium oxalate ($Ca_3(Po_4)_2$ 0·01) respectively.

Injections. Ether anaesthesia was used throughout and all injections were made into the tail vein. Two techniques were used: (1) single injections in which the dose was administered at a steady rate over a period of 5–10 sec, or (2) chronic infusions in which the dose was administered at the rate of 0·05 ml. each 20 sec over a total period of 2–80 min.

Special types of injection were used where mentioned in the text. (1) Freshly prepared suspensions of calcium oxalate or calcium phosphate in stock sodium oxalate were thoroughly shaken immediately before use and injected intravenously over a period of 5–10 sec. (2) The following procedure was used to investigate the rate at which the calcium oxalate is precipitated in plasma. A sublethal dose of the stock solution of sodium oxalate (0·2 ml./100 g) was injected during a period of 5 sec as already described. The needle, still in the vein, was rinsed quickly with 0·1 ml. of normal saline and then a single tracer dose of ^{47}Ca in 0·1 ml. saline was injected at a specified time (10 or 30 sec, 1, 2, 3, 4, 5 or 6 min) after the dose of sodium oxalate. A blood sample was then rapidly taken from the heart. This blood was then left at room temperature for 2 hr to allow complete precipitation of calcium oxalate, and samples of whole blood and plasma were taken for determinations of radioactivity.

Lethal dose. In the case of the single injections the lethal dose was considered to be the smallest dose which would cause the death of all rats within 5 min. Disodium EDTA and sodium citrate nearly always caused death within 1 min, or else the animals recovered, but sodium oxalate was very variable in its speed of action and deaths occurred over a range of 0·5–5 min. In the chronic injections the lethal dose was considered to be the dose which had been administered up to the time when the rats stopped breathing. The usual course of events was as follows: after a certain time during the chronic infusions, depending on the rate of calcium removal, the rats' breathing became laboured, then erratic and finally stopped; when no further respirations occurred for 30 sec the animal was considered to be dead and the time of the last breath was recorded as the end point—very few animals revived after this stage.

Radioactivity determinations were carried out in a scintillation counter with a well-type sodium iodide crystal. All samples were enclosed in a lead sleeve 5 mm thick to absorb the γ-rays from ^{47}Sc, the daughter isotope of ^{47}Ca.

Packed cell volume of blood samples was determined with the aid of a Hawksley micro-haematocrit centrifuge.

RESULTS

Single injection of disodium EDTA, sodium oxalate and sodium citrate

The relative toxicity of disodium EDTA, sodium oxalate and sodium citrate was assessed by first estimating the lethal doses for each when given by the single injection method. The amount of calcium equivalent to this was then calculated and expressed as the 'calcium equivalent' of the lethal dose in mg Ca/100 g rat. The smaller this value the more toxic is the agent and vice versa. Table 1 shows the calcium equivalents of the lethal doses of disodium EDTA, sodium citrate and sodium oxalate.

Disodium EDTA is four times more toxic than sodium citrate and nearly ten times more toxic than sodium oxalate. However, the addition of a small amount of calcium oxalate precipitate to the sodium oxalate increases its toxicity. On the other hand, calcium phosphate has no effect.

TABLE 1. The lethal doses of disodium EDTA, sodium citrate and sodium oxalate when administered by single intravenous injection

		Calcium equiv. of lethal dose (mg Ca/100 g rat)
Disodium EDTA		0·45
Sodium citrate		1·8
Sodium oxalate		4·2
Sodium oxalate	(CaOX 0·1)	1·2
	(CaOX 0·01)	1·8
	($Ca_3(Po_4)_2$ 0·1)	4·2
	($Ca_3(Po_4)_2$ 0·01)	4·2

TABLE 2. The amount of calcium theoretically chelated or removed by disodium EDTA, sodium oxalate and sodium citrate before death occurs, during chronic infusions at various rates

Rate of Ca chelation or removal (mg/min/100 g)	Calcium chelated or removed before death (mg/100 g) by		
	Disodium EDTA	Sodium oxalate	Sodium citrate
1·2	—	—	5·0
0·6	—	—	6·8
0·48	0·96	3·77	—
0·42	0·95	3·70	—
0·36	0·97	3·60	10·8
0·30	0·98	3·42	—
0·24	1·00	3·20	—
0·22	—	—	16·6
0·18	1·00	3·36	—
0·15	1·02	3·30	—
0·12	1·36	3·16	—
0·09	1·71	2·76	—
0·06	1·76	—	—

Table 2 shows the relation between the theoretical rate of removal of calcium during the chronic infusions (mg/min/100 g) and the total calcium removed by the time the rat had died. These results have been obtained from graphs showing the relation between the rates of calcium removal for chelation and the time to kill for each agent, plotted as in Payne & Sansom (1963). Few rats were used to assess the toxicity of sodium citrate because it was not very toxic and the injection had to be continued for long periods.

The distribution of ^{47}Ca between plasma and whole blood in normal rats and in rats treated with sodium oxalate

When a blood sample was taken from a rat shortly after the intravenous injection of a tracer dose of ^{47}Ca, the radioactivity was confined to the

plasma and did not appear to enter the blood cells even after several hours at room temperature. This suggests an alternative method for determining the packed cell volume (P.C.V.). Indeed, calculations based upon the following formula

$$\frac{\text{counts/ml. plasma}}{\text{counts/ml. whole blood}} = \frac{100}{100-\text{P.C.V. (\%)}}$$

give very similar results to those determined by the orthodox microhaematocrit method (Table 3). However, when a tracer dose of ^{47}Ca was injected intravenously into a rat after the administration of stock sodium

TABLE 3. The relation between the ratio of ^{47}Ca concentration in plasma and whole blood after a single intravenous injection of ^{47}Ca, and the packed cell volume

Rat	P.C.V. (%)	Counts/ml. plasma / counts/ml. whole blood	Calculated P.C.V. (%)*
1	41	1·72	42
2	42	1·73	42
3	43	1·72	42
4	41	1·72	42
5	42	1·73	42
6	42	1·67	40

* Where $\frac{\text{counts/ml. plasma}}{\text{counts/ml. whole blood}} = \frac{100}{100-\text{P.C.V.}}$.

TABLE 4. The relation between the ratio of ^{47}Ca concentration in plasma and whole blood after a single intravenous injection of ^{47}Ca at intervals after administration of sodium oxalate, and the calculated relative amounts of sodium oxalate remaining unprecipitated as calcium oxalate at those intervals

Interval between administration of sodium oxalate and calcium-47 (sec)	(min)	Counts/ml. plasma / counts/ml. whole blood Mean ± S.E.	Y % Mean ± S.E.
10		0·45 ± 0·02	74 ± 3·3
30		0·67 ± 0·03	61 ± 3·1
	1	0·78 ± 0·04	55 ± 2·6
	2	0·95 ± 0·09	45 ± 4·4
	3	1·16 ± 0·12	33 ± 3·7
	4	1·48 ± 0·12	14 ± 1·2
	5	1·61 ± 0·23	7 ± 1·0
	6	1·72 ± 0·02	0

oxalate solution, the calculations did not always hold good. The ratio of the concentration of radioactivity in the plasma to that in the whole blood was not related to the haematocrit in this way, unless the interval between the administration of the sodium oxalate and the tracer was at least 6 min. At all intervals shorter than this the ratio (counts/ml. plasma): (counts/ml. whole blood) was less than 1·72, giving a low value for the haematocrit. The obvious way of accounting for this is to suggest that some radioactivity had been removed from the plasma and spun down with the cells.

Table 4 shows the values of the ratio of (plasma radioactivity):(whole-blood radioactivity) at different intervals between the administration of the standard dose of sodium oxalate and the tracer dose of ^{47}Ca, each value being the mean of five determinations. If oxalate takes an appreciable time to precipitate plasma calcium as calcium oxalate, the amount of ^{47}Ca label in the precipitate will depend on the length of time between the injection of oxalate and the isotope. If the interval is brief, then most of the oxalate will still be free at the time the isotope is injected and the resulting precipitate will be radioactive. Conversely, a longer interval will result in a precipitate relatively free from ^{47}Ca. This suggests a method for calculating the time calcium oxalate takes to be precipitated in the blood plasma after the injection of sodium oxalate. The percentage of the tracer precipitated (say Y %) should be proportional to the quantity of free oxalate remaining at the time of the injection, and as the precipitated calcium oxalate is spun down with the blood cells when the blood is centrifuged for the separation of plasma, the ratio of radioactivity in plasma to that in whole blood will be

$$\frac{\text{counts/ml. plasma}}{\text{counts/ml. whole blood}} = \frac{100-Y}{100-\text{P.C.V.}}$$

As has been explained above, Y is large if the tracer is injected very shortly after the sodium oxalate and the ratio of the radioactivity in plasma to that in whole blood is low. Conversely, if the tracer is not injected until all the sodium oxalate has been removed as calcium oxalate all the radioactivity will remain in the plasma and the ratio of counts in the plasma to counts in whole blood will be in the same proportion as that predicted by the P.C.V. determination. Table 4 shows the values of this ratio and the values obtained for Y by substituting them in the above equation.

DISCUSSION

The basic assumption of this work is that the toxicity of disodium EDTA, sodium oxalate and sodium citrate when administered intravenously is due to their ability to chelate or precipitate plasma calcium. Equivalent amounts of these agents should be equally toxic and when injected intravenously should cause death when the concentration of ionic calcium in the blood is reduced below a certain minimum. It is possible to calculate a theoretical lethal dose for each in terms of calcium-removing power. The mean plasma volume is 6·2 ml./100 g rat and the mean plasma calcium concentration is 9·4 mg/100 ml. (Payne & Sansom, 1963), giving a mean total plasma calcium reserve of 0·49 mg Ca/100 g rat. Death should therefore occur before this quantity of calcium is removed.

Experimentally this is found to be true in the case of disodium EDTA, in which the lethal dose by rapid intravenous injection corresponds to the removal of 0·45 mg Ca/100 g rat. Death usually occurs within 15 sec at this dose level and virtually immediately at any higher dose. Lower doses cause considerable respiratory distress with slow recovery. It is therefore concluded that disodium EDTA is a rapid and effective chelating agent when given by rapid intravenous injection. However, when injected slowly its toxicity is reduced. This might be expected, because the longer time allows sources of calcium other than those contained in the plasma alone to be called into use. Even so, the amount of calcium removed before death remains nearly constant at about 1·0 mg over a range of doses from 0·48 to 0·15 mg/100 g/min. This is twice the amount removed by the acute lethal dose. It only increases when the dose rate is further reduced.

The acute lethal doses of sodium citrate and sodium oxalate are 4 and nearly 10 times respectively that for disodium EDTA. There are three possible reasons for this: (1) the chelates formed are less stable; (2) the citrate or oxalate ions may be metabolized and rendered harmless or (3) there may be a time lag between the injection of the agent and the removal of the plasma calcium. This would apply particularly in the case of oxalate, where the precipitation of calcium oxalate may be delayed because of a supersaturation phenomenon.

The stability of the calcium citrate complex is less than that of calcium EDTA, the logarithms of the stability constants being 4·90 and 10·70 respectively. Moreover, it is well known that citrate is metabolized in the Krebs cycle. These are probably the main reasons for the very low toxicity of sodium citrate when injected slowly; the chelate must be readily broken down and the citrate ion metabolized. It is interesting that in the acute tests sodium citrate was nearly as toxic as disodium EDTA and certainly more toxic than sodium oxalate. However, the toxicity falls rapidly as the dose rate is lowered. It is therefore concluded that sodium citrate has a fairly high initial chelating power, but that the stability of the chelate formed is low. The free citrate ions thus formed appear to be rapidly detoxicated by the body tissues.

The low acute toxicity of oxalate is partly attributable to a delay in the precipitation of calcium oxalate or to a supersaturation phenomenon which gives the animal time to mobilize more calcium than is available in the blood plasma alone. The experiments with sodium oxalate containing a suspension of preformed calcium oxalate seem to confirm this. Thus the presence of very small amounts of calcium oxalate in the sodium oxalate solution reduces the minimum lethal dose of sodium oxalate from the equivalent of 4·2 to 1·8 mg Ca/100 g. Increasing the amount of

calcium oxalate tenfold only reduces it a little further, to 1·2 mg Ca/100 g. On the other hand, equivalent amounts of calcium phosphate are completely without effect, indicating that the action of the calcium oxalate is specific rather than due to the gross physical action of solid particles in the injection fluid.

The results of the experiments in which ^{45}Ca was injected intravenously at varying intervals after sodium oxalate give further confirmation of the hypothesis that oxalate is less toxic than disodium EDTA because of the time lag in precipitation. It is possible to calculate the length of time involved. Thus it appears that free oxalate ions are still precipitating calcium up to 6 min after the injection. During the period of delay some mobilization of calcium from reserves outside the blood will almost certainly occur. Indeed, in the experiments with disodium EDTA the available calcium was doubled in 6 min. It might therefore be predicted that the acute lethal dose of sodium oxalate would be only twice that of disodium EDTA instead of nearly tenfold, as it actually is. It therefore seems probable that some additional process other than precipitation removes oxalate ions from plasma. If this process is faster the higher the concentration of oxalate, then we might explain the other curious feature of sodium oxalate toxicity, that it increases with decreasing dose rate. Such a process might depend on renal excretion. The faster the dose rate the higher the plasma concentration of oxalate and the larger the proportion of the dose excreted by the kidney before the oxalate is precipitated as calcium oxalate. At lower dose rates a larger proportion of the dose may be involved in precipitating calcium as calcium oxalate. The conditions under which an acute dose of sodium oxalate is most toxic is in the presence of preformed calcium oxalate, which speeds up the process of precipitation and so prevents the kidney excreting such a high proportion of the dose.

It may be concluded that disodium EDTA is a completely effective agent for the chelation of plasma calcium *in vivo*. Sodium oxalate is fairly effective if administered at low dose rates, but less effective when administered rapidly. Sodium citrate is only effective if administered rapidly and becomes almost non-toxic at low dose rates. Evidence will be presented in another paper of the routes of excretion of disodium EDTA and sodium oxalate.

SUMMARY

1. The relative efficacy of disodium EDTA, sodium oxalate and sodium citrate for inducing experimental hypocalcaemia has been determined by comparing their toxicity to rats when administered intravenously.

2. Disodium EDTA is highly toxic and appears to chelate plasma calcium rapidly and effectively.

3. Sodium oxalate is much less toxic, but its toxicity is specifically increased by the presence of preformed calcium oxalate crystals.

4. Radioisotope experiments indicate that sodium oxalate requires about 6 min to precipitate its equivalent of calcium as calcium oxalate completely.

5. Sodium citrate is highly toxic when given by rapid intravenous injection but its toxicity rapidly declines as the rate of administration is reduced.

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TREATMENT OF SEVERE PUSTULAR DERMATOSES AND STAPHYLOCOCCIC SEPTICEMIA BY ORAL ADMINISTRATION OF PENICILLIN

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IN THE following pages we report 2 severe cases of pyogenic dermatitis. The first case was one of a severe pyoderma of both hands, which was treated by the oral administration of penicillin following two relapses after sulfadiazine therapy. The second case was one of extensive pustular dermatitis, involving the hands, arm and legs. This case was complicated by a hemolytic staphylococcic septicemia.

REVIEW OF EXPERIMENTAL WORK

The administration of penicillin by mouth has been ineffective due to the destruction of the antibiotic substance by the action of hydrochloric acid. This observation was made first by Abraham and Florey and their associates [1] and was subsequently verified by other investigators.[2] This was also proved clinically by Rammelkamp and Helm,[3] who administered penicillin by mouth to 2 patients with pernicious anemia and achlorhydria. Therapeutic blood levels were obtained in these patients. More recent efforts [4] have been made to overcome this major obstacle of inactivation by the hydrochloric acid, and most encouraging results have been achieved. The work of György [5] and his associates was done by

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using penicillin calcium orally with trisodium citrate as a buffer, as suggested by Charney, Alburn and Bernhart.[6] Penicillin is most stable between p_H 4.8 and 7.9 and is inactivated beyond this range.

REPORT OF CASES

CASE 1.—The patient (J. P.), a white man aged 34, was first seen May 8, 1945 while still in bed convalescing from an infection of the upper respiratory tract, which he had had for a week. His temperature and pulse rate were normal, and his general appearance good. His past history indicated that he had had apparent episodes of dyshidrosis. He presented many pustular lesions, ranging in size from 1 mm. to 15 mm. in diameter. Many of these lesions were excavating in character and covered extensive areas of the fingers, palms and backs of both hands. The lesions were tense, some were broken and oozing, and his hands were swollen and red. No visible lymphangitis was present, but axillary lymph nodes could be felt on palpation. Sulfadiazine in doses of 3 Gm. per day, fluids, rest in bed and 1:2,000 solution of mercury bichloride wet packs were prescribed. The lesions gradually dried up and apparent cure took place in eight days, at which time no evidence of even the smallest pustules could be seen.

Four days later there was a decided recurrence of lesions and visible lymphangitis was seen, extending halfway to one elbow. Hospitalization with adequate parenteral penicillin therapy was considered, but because of economic considerations the original treatment was renewed. Within a week all lesions had completely dried up and again what appeared to be a complete recovery had taken place. In view of the first relapse, it was thought advisable to supplement this therapy with penicillin. The patient remained in his home and was given 20,000 units of penicillin intramuscularly every four hours during the day for two days, with a total dosage of 200,000 Oxford units.

Five days later there was complete recurrence of lesions on the fingers, palms and backs of his hands, with multiple lymphangitis extending up both forearms to the elbows. The patient had a noticeable pallor; his temperature was 102 F., and his pulse rate was 120 per minute. Further sulfadiazine therapy was deemed inadvisable, and hospitalization with adequate parenteral penicillin therapy was contemplated.

It was at this point that the oral administration of penicillin was decided on. This was done for several reasons: First, penicillin therapy is effective and safe in the treatment of pyogenic dermatoses.[7] Second, the parenteral administration of penicillin involves a rather elaborate and time-consuming procedure, of which hospitalization is usually a prerequisite. Third, oral therapy is easier and less disagreeable from the patient's standpoint. Fourth, recent studies show that the oral administration of penicillin has real promise in the effective treatment of infectious diseases.

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The patient was given penicillin calcium combined with sodium citrate[8] in the dosage given in the following paragraph. The approximate expected blood levels of penicillin following its oral administration had previously been determined by one of us (H. G. N.)[9] on a small series of patients. At the time the penicillin was given cultures were taken from the lesions on the patient's hands. Staphylococcus aureus and beta hemolytic streptococcus were isolated. The strain of staphylococcus was coagulase positive. Blood cultures taken prior to the administration of the penicillin were negative.

The patient was given 10 penicillin calcium tablets (200,000 units) every hour for four doses or until 40 tablets had been taken. This was followed by 7 tablets every two hours for the first twenty-four hours. Thereafter 5 tablets were given every two hours during the daytime for the following fourteen days.

The results were altogether gratifying. Within eighteen hours after the initial oral dose of penicillin the patient's temperature had dropped from 102 to 98.6 F., and his pulse rate had dropped from 120 to 92 per minute. His general appearance was much improved, and the tense, swollen character of his hands had subsided. The patient also stated that he was feeling much better. At this same time the penicillin blood level was found to be 2.4 units per cubic centimeter of serum, following the method of Cooke.[10] This level is well above the therapeutic range of 0.02 to 0.2 units per cubic centimeter of serum as given by Kolmer.[11]

Owing to the decided clinical improvement, further penicillin blood levels were not ascertained. The patient steadily improved, and the temperature and pulse remained normal. No toxic effects of penicillin were noted. The pustular appearance of the lesions completely disappeared within a week, followed by subsequent gradual sloughing of crusts. Twenty-one days have elapsed since the patient has had any therapy, and he shows no evidence of recurrence of the eruption.

Case 2.—The patient (A. W.), a 20 year old white woman, was admitted to the isolation ward of St. Margaret's Hospital, Kansas City, Kan., at 8 p. m. on June 13, 1945. On admission, examination revealed that the patient had pustular lesions covering extensive areas of the hands, arms, legs and thighs. These varied in size from 1 mm. to 15 mm. in diameter. Many of the pustules had coalesced, forming larger confluent lesions. Typical lesions are seen in figure 1.

The past history revealed that the patient had been working in a war plant for the past several months. She had been wearing clothing laundered with fire-protective processing, and about two weeks prior to the present illness a contact dermatitis developed on her extremities. The pustular lesions appeared three days prior to her admission to the hospital. While being transported in an ambulance from a nearby city she had several chills.

The patient presented a florid, toxic appearance. Her temperature was 104 F. and her pulse rate was 120 per minute. She was listless. The blood count showed 19,000 white cells with 90 per cent polymorphonuclear leukocytes, 24 of which were nonfilamented. Urinalysis showed 10 to 15 red blood cells and 5 to 10 white blood

8. The penicillin calcium tablets combined with sodium citrate, each tablet containing 20,000 units, were kindly supplied by E. R. Squibb and Sons.

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cells per high power field. A blood culture taken at this time showed a pure culture of hemolytic Staph. aureus. Cultures from the lesions on the skin showed these same organisms and in addition beta hemolytic streptococci.

The patient was given 10 penicillin calcium tablets every hour for four doses. The dose was then reduced to 7 tablets every hour for twenty-four hours. Following this the dose was reduced to 5 tablets every two hours during the daytime only. This regimen was continued for nine days, at which time the medication was stopped.

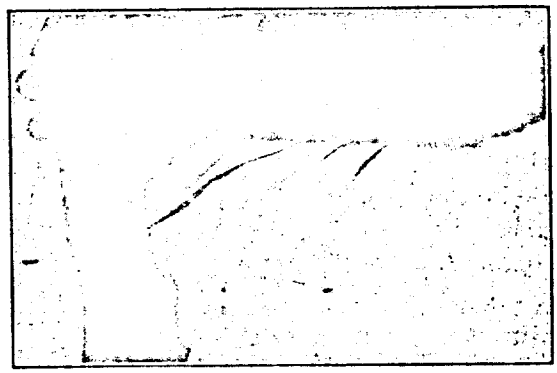

Fig. 1 (case 2).—Pustular lesions on hands and arms. The lesions are equally numerous on the ankles, legs and thighs.

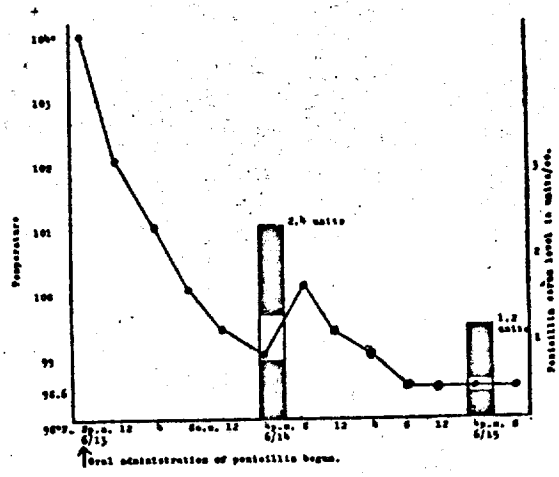

Fig. 2 (case 2).—Simultaneous changes in temperature and in penicillin blood serum levels.

The temperature and penicillin levels are shown in figure 2. It is seen that the temperature dropped from 104 to 99 in eighteen hours. Within thirty-six hours the temperature was 98.6. The penicillin blood level was 2.4 units per cubic centimeter twenty hours after the initial dose was given. The second day after administration the level was 1.2 units, and the levels on the third, fifth and seventh days was 0.2 units per cubic centimeter.

Blood cultures taken twenty-four and seventy-two hours after admission were negative. The white blood count in forty-eight hours had dropped from 19,000, on admission, to 8,000 with 70 per cent polymorphonuclear leukocytes, 14 of which were nonfilamented. Five days after admission the white count was 7,000 with 58 per cent polymorphonuclear leukocytes, 6 of which were nonfilamented.

The patient's general condition showed remarkable improvement within twelve hours after the initial medication was given. By the end of five days after the penicillin was administered the pustules had completely dried up, leaving only scaling, yellow crusts. By the end of seven days the crusts had disappeared. The patient was dismissed from the hospital on June 23, 1945, ten days after admission.

It is our impression that the patients would probably have improved with smaller doses of penicillin than those given.

CONCLUSIONS

1. A patient with severe pustular dermatitis was treated successfully by the oral administration of penicillin, following two relapses with sulfadiazine therapy and intramuscular administration of penicillin.

2. A patient with severe generalized pustular dermatitis complicated by a hemolytic staphylococcic septicemia responded dramatically to the oral administration of penicillin.

3. No toxic effects from the penicillin were noted with the dosages given.

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for the action of ionizing rays on the formation of antibodies and plasma proteins. Additional investigations in connection with the role of the adrenal in bringing about these phenomena are in progress.

Conclusions

1. The formation of antibodies (anti-proteus $X_{19}O$ agglutinins and anti-sheep haemolysins) is inhibited by irradiation performed two days prior to the primary immunizing injection (200 r) or 11 days prior to the secondary injection (150 and 300 r). This effect is proportionate to the dose of irradiation. Irradiation with 200 r applied two days after the first immunization no longer influences immunogenesis.

2. Plasma protein levels (total proteins or fractions) displayed no changes that might be ascribed to irradiation. The different changes observed may only be related to the moment the antigens were injected and to repeated bleeding. The lack of any apparent changes related to irradiation may be accounted for by their being masked owing to simultaneous changes in opposite directions of the various protein fractions in the plasma.

3. Irradiation with 150—300 r does not alter blood complement concentration in rabbits. Characteristic changes are present only in relation to the appearance of antibodies, but the direction of these changes differs, depending on whether irradiation inhibits the immune response or fails to do so.

4. The absence of changes in proteinaemia corresponding to differences in immune reactions between rabbits irradiated during the radiosensitive phase and controls, was explained in the same manner as the complement changes, i.e. by the intervention of a regulation mechanism based on the existence of a physical-chemical balance between the plasma concentration and the cellular concentration of each protein fraction.

Sodium citrate, pharmacodynamic action.

CONTRIBUTIONS TO THE STUDY OF THE PHARMACODYNAMIC ACTION OF SODIUM CITRATE; THE REFLEX PHARMACODYNAMIC ACTION OF THIS SUBSTANCE

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In a previous paper the authors have shown that sodium citrate administered by the intravenous route alters the vascular tonus, contracts the spleen, and stimulates the respiratory centre. These influences are the expression of the direct pharmacodynamic action of this substance. The authors demonstrate that in addition to this direct action, citrate exerts a reflex chemoreceptive action as well.

The experiments were carried out in dogs, by perfusing organs *in situ*, which were isolated from the vascular point of view but maintained intact nervous connections. Either the organ thus isolated (spleen, intestine, etc.), or the entire body (minus the respective organ) were used as reflexogenic zones, i.e. sodium citrate was alternately injected either into the perfusion fluid (at the entrance into the organ) or into the systemic circulation (intravenously).

Conclusions

Sodium citrate stimulates the chemoreceptors and determines the appearance of a series of reflexes:

1. A respiratory reflex: the respiratory movements increase both in intensity and frequency.

2. A pressor reflex of the compensatory type; due to this reflex, the hypotension induced by this substance through its direct action is partly checked.

3. An intestinal reflex of the inhibitory type: peristalsis and the intestinal tonus are considerably decrased.

4. A splenic reflex: the spleen contracts. Hence, the reflex action of sodium citrate on the spleen is of the same kind as the direct action. There is thus summation of both actions of the substance. During transfusions with citrated blood, contraction of the spleen and evacuation of the blood stored in this organ will inevitably take place. Hence, not only the injected blood, but also stored blood still available are involved in the restoration of the blood volume.

Antimalarial drugs. Spleen, reflexes.

CONCERNING THE PHENOMENA OF INHIBITION AND ADAPTATION OF INTEROCEPTIVE REFLEXES TO THE ACTION OF ANTIMALARIAL DRUGS

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(Medical Institute, Jassy. Laboratory of Pharmacology. Director: Prof. Gh. Popovici)

The present study deals with the results of our investigations on two phenomena, i.e. inhibition of the interoceptive reflexes and adaptation to this inhibiting action under the influence of atabrine, chloroquine, quinine, and paludrine applied to the end of the reflex arc, at the level of the interoceptors.

The investigations were carried out in an acute experiment, in about 170 dogs, by perfusing the spleen or an intestinal loop isolated from the general blood circulation but with maintenance of the nervous connections according to the technique employed by *Bykov*, *Tchernigovski* and co-workers, as modified by *Popescu*. The action of antimalarial drugs on respiratory, circulatory, and in some cases, intestinal interoceptive reflexes start-

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Effect of Sodium Citrate on the Growth of Transplantable Tumors

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In studying the effect, on a tumorous growth, of serum or of the cellular elements of blood, it is constantly necessary to utilize substances that inhibit the coagulation of the blood (heparin, sodium citrate), or those that are instrumental in dividing its fractions (trilon B and others). In carrying out such investigations, we turned our attention to the fact that the indicated substances are not indifferent with respect to the growth of the tumor.

According to the data in the literature, it is known that heparin can manifest an effect on the growth and metastatic sprad of a tumor, even though there does not exist a single opinion with regard to the character of this effect. Thus, in conformity with the data of some authors (6, 9 - 13, 7, and others), heparin inhibits the growth of transplantable tumors, and, in the case of administration together with a suspension of tumorous tissue, diminishes the number of cells that have "taken". Other researchers (8 and others) believe that heparin intensifies the growth of the transplantable tumors. In the opinion of Sakrzewski (14), heparin is instrumental in the metastatic spread of a tumor. The data of various authors, with regard to the effect of heparin on a tumorous growth, have been set forth in the survey of V. Ya. Shats (5).

The unfavorable effect of heparin, for the development of a tumor, is postulated depending on its anticoagulating effect (13, 14, and others), or else, it is explained by the increase in the protective forces of the organism (1).

The effect, on the growth of the tumor, of other substances, which exert an influence on the coagulating system of the blood, has been studied to a lesser

degree. Goerner and Island (10) have noted the action of sodium oxalate in inhibiting the growth of a tumor; I. F. Grekh and V. Ya. Shats (2) have shown that a prolonged administration of phenylene and of neodicoumarin stimulates the growth and metastatic spread of a transplantable tumor.

We studied the effect, on the transplantability and growth of a Walker carcinosarcoma and of a Pliss lymphysarcoma, of preliminarily incubating them in solutions of sodium citrate, heparin, and trilon B.

In the first series of experiments, the tissue suspension of a Walker carcinosarcoma, prepared by the customary method for transplantation, was divided into 2 parts. One of them was kept for 2 hours at a temperature of 37 deg C in a 3% solution of sodium citrate in a ratio of 7 parts, by volume, of the tumorous tissue to 1 part of the solution of sodium citrate; and the other -- under the same conditions, in a physiological solution. Each of these portions was transplated in 20 rats. The dimensions of the tumor were measured with callipers after a day. The volume of the tumor was calculated, taking into consideration its shape; at the same time, in each section of the tumor, a measurement was made of the greatest possible number of its diameters. At the end of the experiments, the animals were killed, the tumor was weighed, and its volume was determined by means of immersion in a cylinder withliquid. The data that were obtained are set forth in composite Table 1.

In Fig. 1, the results of this series of experiments are set forth in the form of kinetic curves in accordance with the method proposed by N. M. Emanuel and A. P. Lingina and approved in subsequent papers (3, 7). All the curves have been plotted by making use of parameters that had been calculated from the experimental data with each individual preparation. As may be seen from the numerical data and from the curves set forth, a tumor, that had been kept in the citrate, grew considerably more slowly than in the case of the control ($X = 1.64$). In spite of the fact that in this experiment, the citrate was added in smaller doses than it is customarily added for blood stabilization, its inhibiting effect on the growth of the tumor was clearly expressed.

In the following series of experiments, we made a study of the action of sodium citrate, in various dilutions, on the transplantability and growth of a Pliss lymphosarcona. The pulverized tumorous tissue was divided into 6 parts; the first one was mixed with a 3% solution of sodium citrate in a ratio of 5 parts, by volume, of the pulverized tumorous tissue, to 1 part of the solution of sodium citrate; the 2nd one - with an identical volume for the solution of sodium citrate 91: 1); the 3rd one, in a ratio of 1 part of the tumorous tissue to 5 parts of a solution of sodium citrate. The fourth, fifth and sixth portions of the pulverized tumorous tissue were mixed with a physiological solution in the same ratios as in the case of the 1st, 2nd and 3rd portions with a solution of sodium citrate (control experiments). Each portion was kept for 30 minutes in a thermostat at a temperature of 37 deg C and was transplanted in 20 rats with the calculation that each animal should receive 0.2 milliliter of the tumorous tissue.

((Three graphs on pg. 79 of text)) ((underneath each graph, reading from left to right)): Days; Fig. 1; Days; Fig. 2; Days; Fig. 3. ((below these graphs, reading down on the left side and then going to the extreme right)):

Fig. 1. Averaged kinetic curves for the growth of a Walker carcinosarcoma in the control (circle) and in the experiment (square) on adding sodium citrate. Fig. 2. Averaged kinetic curves for the growth of a Pliss lymphosarcoma in the control (circle) and in the experiment (square) on adding sodium citrate. Fig. 3. Averaged kinetic curves for the growth of a Pliss lymphosarcoma in the control (circle) and in the experiment on adding sodium citrate (square) or heparin (triangle)

((Table on pg, 80 of text)) ((see the next page for this table))

Table 1

Transplantability and Development of a Tumor in Rats Under Various Influences

No. of Series	Form of Tumor	What was Administered	Periods of Investigation, in days	Number of rats with Tumors: Control	Number of rats with Tumors: Experiment	Volume of Tumor in Millimeters: Control	Volume of Tumor in Millimeters: Experiment	Inhibition	Control	Experimental	Inhibition	% of Inhibition	Index of Growth Inhibition of the Tumor
I	Walker carcinosarcoma	Sodium Citrate 1:7	5	19	19	0.2±0.03	0.03±0.02	5.1					
			10	20	19	2.8±0.6	0.3±0.06	6.2					
			15	20	20	15.2±2.6	9±1.1	2.3	17.2±2.1	10.1±1.8	2.6	41	1.7
II	Pliss lymphosarcoma	Sodium Citrate 1 : 5	5	18	7	0.25±0.02	0.05±0.01	8					
			10	20	14	16.8±1.4	6.1±1.2	5.8					
			15	20	19	66±4	27.1±5.6	6.4	39.5±2.5	16.6±2.5	3.7	58	2.4
		Sodium Citrate 1 : 1	15	20	0	66±4	-		39.5±2.5	-		100	
		Sodium Citrate 5 : 1	15	20	0	66±4	-		39.5±2.5	-		100	
III		Trilon 1 : 1	5	10	7	0.5±0.08	0.2±0.02	3.7					
			10	15	13	5.9±1	0.8±0.06	5					
			15	15	15	15.3±1.3	4.9±0.3	8	19.6±2.3	6.7±1.3	4.9	66	2.9
		Heparin 25,000 mouse un.	5	10	15	0.5±0.08	0.8±0.1	2.3					
			10	15	15	5.9±1.0	4.07±0.25	1.7					
			15	15	15	15.3±1.3	11.6±1.2	2.1	19.6±2.3	15.4±2	1.4	22	1.37
IV		Heparin 5000 mouse units	15	15	15	40±2.7	20.1±4.5	3.8	37.6±3.5	22.4±4.2	2.8	49	1.7
			15	15	15	50±2.7	12±1.3	9.3	37.6±3.5	17.4±2.2	4.3	54	2.2
		Medical Control Norm 1 : 3											

Remark. In the I and II series, in each group, there were 20 rats; in III and IV -- 15 rats.

((text continued)): The mixing of the suspension of pulverized lymphosarcoma tissue with a 3% solution of citrate, in a ratio of 5 : 1 and 1 : 1, made the tumor cells incapable of further growth. Not in a single one of the animals in these two groups did the tumor "take", even though in the control, the same dilution of the tumorous tissue suspension by means of a physiological solution did not exert any influence on the growth of the tumor and its transplantability was 100%.

In diluting 1 part, by volume, of a solution of sodium citrate with 5 parts of a suspension of lymphysarcoma tissue, the tumor "took" in the case of all the animals, but with a considerable retardation of its growth in comparison with the control, in periods and in dimensions (see Table 1). The growth dynamics of the tumor in the control and experimental groups of animals is reflected in the form of the kinetic curves in Fig. 2. As may be seen from the data cited, a Pliss lymphosarcoma proved to be no less sensitive to the action of sodium citrate than a Walker carcinosarcoma.

In the third series of experiments, we made a study of the action on a lymphosarcoma of heparin and trilon B (ethylenediaminetetraacetic acid), which did not reduce the coagulability of the blood, but is frequently employed for a rapid and complete separation of blood fractions). The suspension of lymphosarcoma cells, which had been prepared for transplantation, was divided into 3 parts; the first one was kept in a 3% solution of trilon B in a ratio of 1 : 1; the second one -- in the same ratio, in heparin, with an activity of 25,000 muscle units in 1 milliliter; and the third one (the control) -- in a physiological solution. The experimental conditions were just the same as in the preceding series. Incubation in heparin produced, in the later stages, some inhibition in the growth of the tumor, even though, in the case of treatment by the variation-statistical method, this distinction was not reliable (T = 1.4). In the initial growth period, in the rats of this group, tumors were palpable sooner and were somewhat more sizable than those in the control.

Incubation in a solution of trilon B produced a later development of the tumor and a retardation of its growth in comparison with the control over the entire extent of the experiment (see Table 1).

In the last series of experiments we compared the action of heparin and sodium citrate on the growth of a lymphosarcoma. The heparin utilized in this series of experiments was of a lower concentration than the one utilized in the preceding series -- 5000 muscular units.

In the case of rats in which had been transplanted a suspension of tumorous cells, after maintenance in heparin, tumors were palpable sooner,and in the first 5 - 10 days, they grew more rapidly than those in the control, the same as in the preceding series of experiments. In the subsequent days, the growth of tumors in the experimental group of animals lags behind that in the control (group). Following incubation in sodium citrate, the tumor grew more slowly than theone in the control, over the entire extent of the experiment (see Table 1 and Fig. 3).

For the convenience of comparing the results obtained in all the above-mentioned series of experiments, we have compiled a composite Table 2, representing in it the percent of growth inhibition of the tumor relative to the control, at the end of the experiment, and converting the concentration of the substances under study to 1 gram of the tumorous suspension.

As may be seen from Table 2, sodium citrate, which has been added in vitro to the tissue suspension of a Walker carcinosarcoma and of a Pliss lymphosarcoma, inhibits the growth of the tumors; at the same time, in a concentration of 30 milligrams/gram of the tumorous suspension and above, it brings about the destruction of the tumorous cells.

In reducing the concentration of the sodium citrate, its action weakens. Trilon B inhibits the growth of the tumor but to a somewhat lesser extent. The inhibiting action of these preparations is less frequently pronounced during the early stages in the growth of the transplantable tumors. Heparin, under the same conditions, possesses a less definite action on the cells of a lymphosarcoma; at the same time, in our experiments, heparin of low activi-

ty produced a more considerable inhibition in the growth of the tumor than did heparin of high activity. In both series of experiments, in rats in which there had been transplanted a tumorous tissue following incubation in heparin, the tumors started to be palpable sooner, and in the first days, they grew more rapidly than those in the control; this, afterwards, was replaced by a slower development of the tumor.

((Table on pg. 82 of text))

Table 2

Inhibition in the Growth of the Tumor Depending on the Concentration of the Substance Under Study

Tumor	Substance Under Study	Amount of the Substance for 1 Gram of the Tumorous Suspension	% of Inhibition in the Growth of the Tumor
Walker Carcinosarcoma-256	Sodium Citrate	4.3 microns	41
Pliss Lymphosarcoma	The same	6 milligrams	54
The same	" "	10 "	58
" "	" "	30 "	100
" "	" "	130 "	100
" "	Trilon B	30 "	66
" "	Heparin	25,000 mouse units	22
" "	"	1666 mouse units	49

((text continued)): As has been shown previously (4), sodium citrate, on administering it intravenously in a single dose of 250 milligrams/kilogram (for a course of 1 gram/kilogram) likewise inhibits the growth of a Walker carcinosarcoma; at the same time, a less concentrated solution (5%) manifests a more pronounced effect than does one of 30%. The mechanism of this effect remains unclear to us. It may be assumed that at the basis of the action of the substances under study lies not only their anticoagulation activity, but also theinfluence on metabolic processes. According to our observations, an inhibition in the growth of the transplanted tumors may be produced not only by means of sodium citrate, but also by other substances that enter into the St. Dierdy-Krebs citrate cycle, for example, by means of succinic acid.

A continuation of studying the influence of sodium citrate on the growth of malignant tumors seems to us to be particularly important for the reason that this preparation is widely employed in the oncological clinic as a blood stabilizer and one of the ingredients in blood-substitution liquids.

The possibility of utilizing sodium citrate by means of venoclysis (intravenous infusion) in considerable single doses (up to 250 milligrams/kilogram) has been approved clinically; thus, in preventing the antiblastic effect of sodium citrate, this preparation may prove to be valuable for oncological practice.

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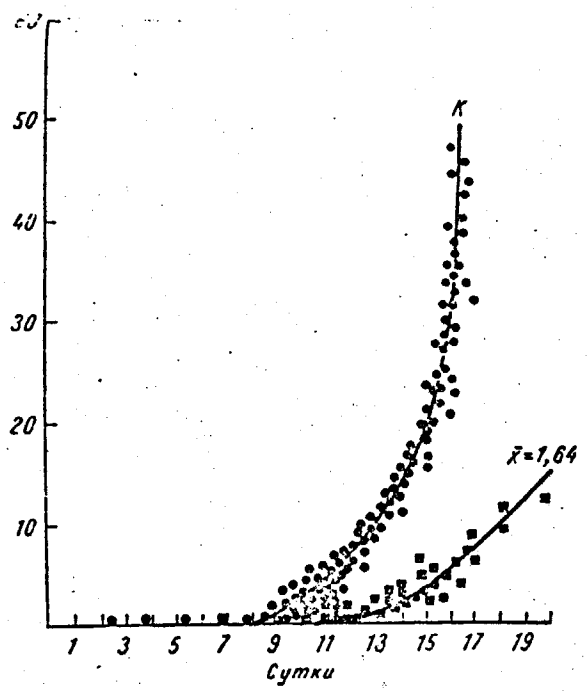

Figure 1

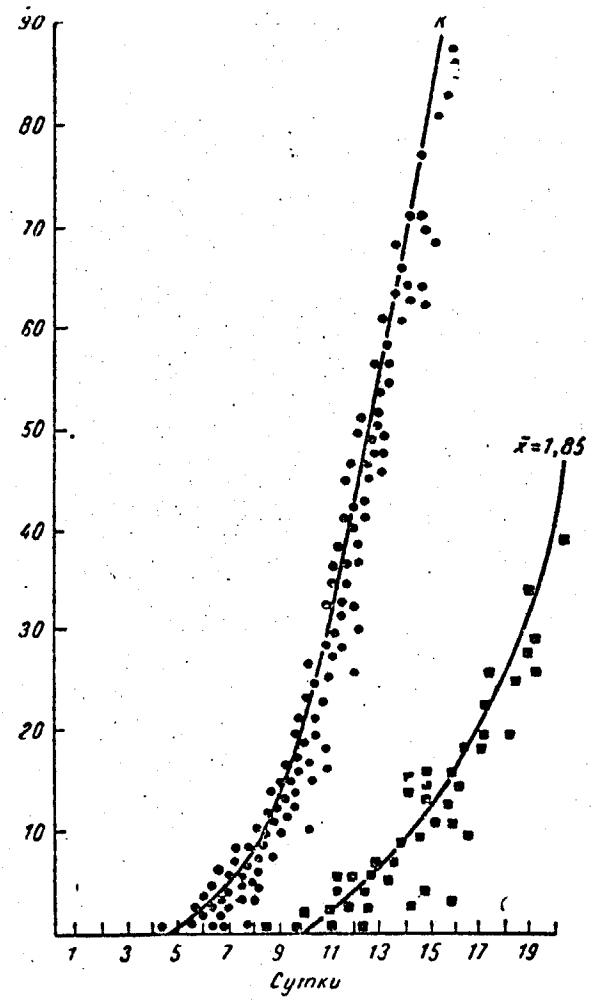

Figure 2

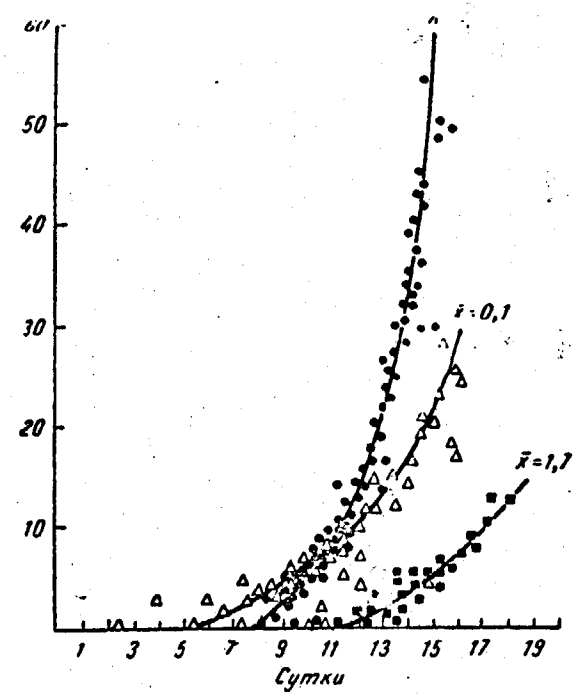

Figure 3

sodium lactate citrate

3. Колебательный характер содержания белка зависит от истинных колебаний его содержания в клетке, а также от перераспределения клеток с различным содержанием белка.

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THE PROTEIN CONTENT IN CELLS OF THE RAT OVARY ASCITIC TUMOR (STRAIN OYA)

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There were determined two periods in tumor development: the first period from the 3dto 10th day, characterized by daily variations in the protein content and concentration, and the second — from the 10th to 14th day when the disturbances in this regularity are observed. The cell attains its maximum size on the 7th and 12th day after transplantation and minimum — on the 10th — and 14th day of the tumor development. A conclusion is made on rhythmical development of tumor, its considerable chemical heterogeneicity and the relationship between the protein content alterations, its virtual changes in the cell and redistribution of cells with different protein content.

ВОПРОСЫ ОНКОЛОГИИ, ТОМ XV, № 3, 1969.

УДК 616-006-092.9-02 : 546.33

О ВЛИЯНИИ ЛИМОННОКИСЛОГО НАТРИЯ НА РОСТ ПЕРЕВИВАЕМЫХ ОПУХОЛЕЙ

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При изучении влияния на опухолевый рост сыворотки или клеточных элементов крови постоянно приходится применять вещества, пре-

пятствующие свертыванию крови (гепарин, лимоннокислый натрий) или способствующие разделению ее фракций (трилон Б и др.). При выполнении таких исследований мы обратили внимание на то, что указанные вещества не являются безразличными для роста опухоли.

По литературным данным, известно, что гепарин может оказывать влияние на рост и метастазирование опухоли, хотя единого мнения о характере этого влияния не существует. Так, согласно данным одних авторов [6, 9—13, 7 и др.], гепарин подавляет рост перевиваемых опухолей и при введении вместе со взвесью опухолевой ткани уменьшает количество приживающих клеток. Другие исследователи [8 и др.] считают, что гепарин усиливает рост перевиваемых опухолей. По мнению Zakrzewski [14], гепарин способствует метастазированию опухоли. Данные различных авторов о влиянии гепарина на опухолевый рост приведены в обзоре В. Я. Шац [5].

Неблагоприятное для развития опухоли действие гепарина ставится в зависимость от его антикоагулирующего эффекта [13, 14 и др.] или объясняется повышением защитных сил организма [1].

Действие на рост опухоли других веществ, влияющих на свертывающую систему крови, менее изучено. Goerner и Island [10] отметили тормозящее рост опухоли действие оксалата натрия, И. Ф. Грех и В. Я. Шац [2] показали, что длительное введение фенилена и неодикумарина стимулирует рост и метастазирование перевиваемой опухоли.

Мы изучали влияние на перевиваемость и рост карциносаркомы Уокера и лимфосаркомы Плисса предварительной инкубации их в растворах лимоннокислого натрия, гепарина и трилона Б.

В первой серии опытов приготовляемую обычным методом для перевивки взвесь ткани карциносаркомы Уокера разделяли на 2 части. Одну выдерживали 2 *ч* при температуре 37° С в 3% растворе лимоннокислого натрия в соотношении 7 объемных частей ткани опухоли к 1 части раствора лимоннокислого натрия, другую — в тех же условиях в физиологическом растворе. Каждую из этих порций перевивали 20 крысам. Размеры опухоли измеряли через день кронциркулем. Объем опухоли вычисляли с учетом ее формы, причем в каждом участке опухоли измеряли возможно большее число ее диаметров. В конце опытов животных убивали, опухоль взвешивали, и объем ее определяли путем погружения в цилиндр с жидкостью. Полученные данные представлены в сводной табл. 1.

На рис. 1 результаты этой серии опытов представлены в виде кинетических кривых по методу, предложенному Н. М. Эмануэлем и А. П. Лишиной и апробированному в последующих работах [3, 7]. Все кривые построены с использованием параметров, вычисленных из данных опыта с каждым отдельным препаратом. Как видно из цифровых данных и приведенных кривых, опухоль, выдержанная в цитрате, росла значительно медленнее, чем в контроле ($X=1{,}64$). Несмотря на то, что в этом опыте цитрат добавлялся в меньших дозах, чем обычно добавляется для стабилизации крови, тормозящий эффект его на рост опухоли был четко выражен.

В следующей серии опытов мы изучали действие лимоннокислого натрия в различных разведениях на перевиваемость и рост лимфосаркомы Плисса. Измельченная ткань опухоли была разделена на 6 частей, первая смешивалась с 3% раствором лимоннокислого натрия в соотношении 5 объемных частей измельченной опухолевой ткани к 1 части раствора лимоннокислого натрия, 2-я — с одинаковым объемом раствора лимоннокислого натрия (1 : 1), 3-я в соотношении 1 часть опухолевой ткани к 5 частям раствора лимоннокислого натрия. Четвертая, пятая и шестая порции измельченной опухолевой ткани смешивались с физиологическим раствором в тех же соотношениях, как 1, 2 и 3-я порции с раствором лимоннокислого натрия (контрольные опыты). Каждая порция выдерживалась 30 *мин* в термостате при

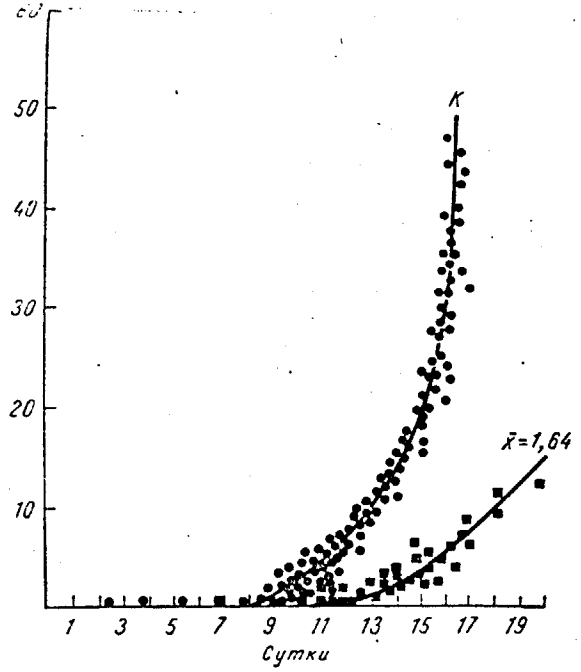

Рис. 1.

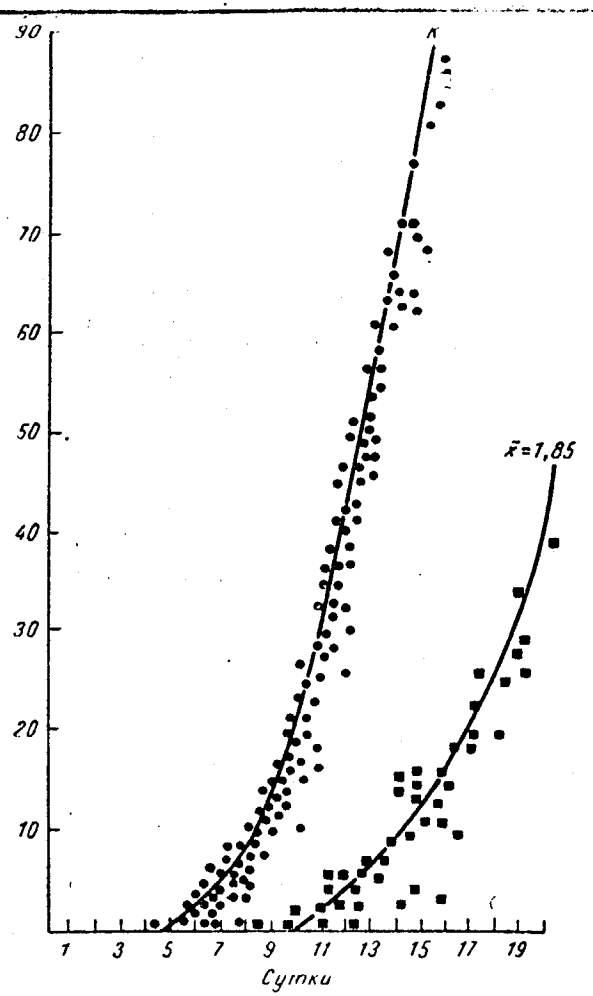

Рис. 2

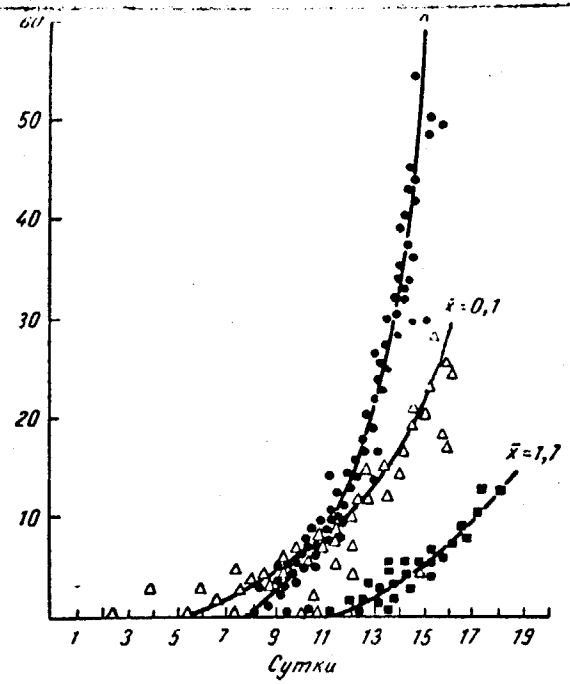

Рис. 3

Рис. 1. Усредненные кинетические кривые роста карциносаркомы Уокера в контроле (кружок) и в опыте (квадрат) при добавлении лимоннокислого натрия.

Рис. 2. Усредненные кинетические кривые роста лимфосаркомы Плисса в контроле (кружок) и в опыте (квадрат) при добавлении лимоннокислого натрия.

Рис. 3. Усредненные кинетические кривые роста лимфосаркомы Плисса в контроле (кружок) и в опыте при добавлении лимоннокислого натрия (квадрат) или гепарина (треугольник).

Таблица 1

Перевиваемость и развитие опухоли у крыс при различных воздействиях

№ серии	Вид опухоли	Что введено	Сроки исследования в днях	Число крыс с опухолями		Объем опухоли в *мл*			Средний вес опухоли в *г*			% торможения	Индекс торможения роста опухоли
				К	О	К	О	Т	К	О	Т		
I	Карциносаркома Уокера	Лимонно-кислый натрий 1 : 7	5	19	18	0,2±0,03	0,03±0,02	5,1					
			10	20	19	2,8±0,6	0,3±0,06	6,2					
			15	20	20	15,2±2,6	9±1,1	2,3	17,2±2,1	10,1±1,8	2,6	41	1,7
II	Лимфосаркома Плисса	Лимонно-кислый натрий 1 : 5	5	18	7	0,25±0,02	0,05±0,01	8					
			10	20	14	16,8±1,4	6,1±1,2	5,8					
			15	20	19	66±4	27,1±5,6	6,4	39,5±2,5	16,6±2,5	3,7	58	2,4
		Лимонно-кислый натрий 1 : 1	15	20	0	66±4	—		39,5±2,5	—		100	
		Лимонно-кислый натрий 5 : 1	15	20	0	66±4	—		39,5±2,5	—		100	
III		Трилон 1 : 1	5	10	7	0,5±0,08	0,2±0,02	3,7					
			10	15	13	5,9±1	0,8±0,06	5					
			15	15	15	15,3±1,3	4,9±0,3	8	19,6±2,3	6,7±1,3	4,9	66	2,9
		Гепарин 25 000 мыш. ед.	5	10	15	0,5±0,08	0,8±0,1	2,3					
			10	15	15	5,9±1,0	4,07±0,25	1,7					
			15	15	15	15,3±1,3	11,6±1,2	2,1	19,6±2,3	15,4±2	1,4	22	1,37
IV		Гепарин 5000 мыш. ед. ЛКН 1 : 3	15	15	15	40±2,7	20,1±4,5	3,8	37,6±3,5	22,4±4,2	2,8	49	1,7
			15	15	15	50±2,7	12±1,3	9,3	37,6±3,5	17,4±2,2	4,3	54	2,2

Примечание. В I и II сериях в каждой группе было по 20 крыс; в III и IV — по 15 крыс.

температуре 37° С и перевивалась 20 крысам с таким расчетом, чтобы каждое животное получило 0,2 *мл* опухолевой ткани.

Смешивание взвеси измельченной ткани лимфосаркомы с 3% раствором цитрата в соотношении 5 : 1 и 1 : 1 делало клетки опухоли неспособными к дальнейшему росту. Ни у одного из животных этих двух групп опухоль не привилась, хотя в контроле такое же разведение взвеси опухолевой ткани физиологическим раствором не повлияло на рост опухоли и перевиваемость ее была 100%.

При разведении 1 объемной части раствора лимоннокислого натрия к 5 частям взвеси ткани лимфосаркомы опухоль привилась у всех животных, но со значительным отставанием ее роста от контроля в сроках и размерах (см. табл. 1). Динамика роста опухоли в контрольной и подопытной группах животных отображена в виде кинетических кривых на рис. 2. Как видно из приведенных данных, лимфосаркома Плисса оказалась не менее чувствительной к действию лимоннокислого натрия, чем карциносаркома Уокера.

В третьей серии опытов мы изучали действие на лимфосаркому гепарина и трилона Б (этилендиаминтетрауксусной кислоты, не снижающей свертываемость крови, но часто применяемой для быстрого и полного разделения фракций крови). Приготовленная для перевивки взвесь клеток лимфосаркомы была разделена на 3 части, первая выдерживалась в 3% растворе трилона Б в соотношении 1 : 1, вторая — в том же соотношении в гепарине активностью 25 000 мыш. ед. в 1 *мл*, третья (контрольная) — в физиологическом растворе. Условия опыта были, как и в предыдущей серии. Инкубация в гепарине вызывала на поздних стадиях некоторое торможение роста опухоли, хотя при обработке вариационно-статистическим методом это различие не было достоверным ($T=1{,}4$). В начальном периоде роста у крыс этой группы опухоли прощупывались раньше и были несколько крупнее, чем в контроле.

Инкубация в растворе трилона Б вызывала более позднее развитие опухоли и отставание ее в росте по сравнению с контролем на всем протяжении опыта (см. табл. 1).

В последней серии опытов мы сравнивали действие гепарина и лимоннокислого натрия на рост лимфосаркомы. Применяемый в этой серии опытов гепарин был более низкой концентрации, чем применяемый в предыдущей серии — 5000 мыш. ед.

У крыс, которым перевивалась взвесь опухолевых клеток после выдерживания в гепарине, опухоли прощупывались раньше и в первые 5—10 дней росли быстрее, чем в контроле, так же, как и в предыдущей серии опытов. В последующие дни рост опухолей у опытной группы животных отстает от контрольного. После инкубации в лимоннокислом натрии опухоль росла медленнее, чем в контроле на всем протяжении опыта (см. табл. 1 и рис. 3).

Для удобства сопоставления результатов, полученных во всех вышеописанных сериях опытов, мы составили сводную табл. 2, представив в ней процент торможения роста опухоли по отношению к контролю в конце опыта и пересчитав концентрацию изучаемых веществ на 1 *г* опухолевой взвеси.

Как видно из табл. 2, лимоннокислый натрий, добавленный in vitro к взвеси ткани карциносаркомы Уокера и лимфосаркомы Плисса, тормозит рост опухолей, причем в концентрации 30 *мг/г* опухолевой взвеси и выше он вызывает гибель опухолевых клеток.

При понижении концентрации лимоннокислого натрия действие его ослабевает. Трилон Б также тормозит рост опухоли, но в несколько меньшей степени. Тормозящее действие этих препаратов резче выражено на ранних стадиях роста перевиваемых опухолей. Гепарин в тех же условиях обладает менее определенным действием на клетки лимфосаркомы, причем в наших опытах гепарин низкой активности вызвал

более значительное торможение роста опухоли, чем гепарин высокой активности. В обеих сериях опытов у крыс, которым перевивалась опухолевая ткань после инкубации в гепарине, опухоли начинали прощупываться раньше и в первые дни росли быстрее, чем в контроле, что затем сменялось более медленным развитием опухоли.

Таблица 2

Торможение роста опухоли в зависимости от концентрации исследуемого вещества

Опухоль	Исследуемое вещество	Количество вещества на 1 г опухолевой взвеси	% торможения роста опухоли
Карциносаркома Уокера-256	Лимонно-кислый натрий	4,3 *мк*	41
Лимфосаркома Плисса	То же	6 *мг*	54
То же	» »	10 »	58
» »	» »	30 »	100
» »	» »	130 »	100
» »	Трилон Б	30 »	66
» »	Гепарин	25 000 м. ед.	22
» »	»	1666 м. ед.	49

Как показано ранее [4], лимоннокислый натрий при внутривенном введении его в разовой дозе 250 *мг/кг* (на курс 1 *г/кг*) также тормозит рост карциносаркомы Уокера, причем менее концентрированный раствор (5%) оказывает более выраженное действие, чем 30%. Механизм этого эффекта остается для нас неясным. Можно предположить, что в основе действия изучаемых веществ лежит не только их антикоагуляционная активность, но и влияние на обменные процессы. По нашим наблюдениям, торможение роста перевиваемых опухолей можно вызвать не только лимоннокислым натрием, но и другими веществами, входящими в цитратный цикл Сен-Дьерди — Кребса, например янтарной кислотой.

Продолжение изучения влияния лимоннокислого натрия на рост злокачественных опухолей представляется нам особенно важным потому, что этот препарат широко применяется в онкологической клинике как стабилизатор крови и один из ингредиентов кровезаменяющих жидкостей.

Возможность применения лимоннокислого натрия путем внутривенных вливаний в значительных разовых дозах (до 250 *мг/кг*) апробирована в клинике, таким образом, при подтверждении антибластического эффекта лимоннокислого натрия этот препарат может оказаться ценным для онкологической практики.

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ON THE EFFECT OF SODIUM CITRATE ON THE TRANSPLANTABLE TUMOR GROWTH

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The effect of sodium citrate, heparin and trilon B on growth and transplantability rat tumors the Walker carcinosarcoma and Pliss lymphosarcoma has been studied. mor cell suspension was sustained in the above mentioned substances of various con- tration and the mixture was transplanted onto rats. It was demonstrated that sodium ate reduced the transplantability and inhibited growth of the Walker carcinosarcoma d Pliss lymphosarcoma. The efficacy of the substance is directly proportional to its centration. Trilon B also possesses a similar action. The effect of heparin was found be of a less definite character.

ВОПРОСЫ ОНКОЛОГИИ, ТОМ XV, № 3, 1969 г.

УДК 616-006-02 : 615.849.7

ВЛИЯНИЕ ГАММА-РАДИАЦИИ НА ИНДУКЦИЮ ОПУХОЛЕЙ УЛЬТРАФИОЛЕТОВЫМИ ЛУЧАМИ

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В настоящее время имеется ряд клинических наблюдений и экспе- ментальных данных, показывающих, что одним из этиологических акторов злокачественных новообразований кожи следует считать резмерное воздействие ультрафиолетовых (УФ) лучей [2, 4, 5, 10, 2—14, 17, 18, 23—28, 31]. Необходимым условием возникновения зло- чественного роста под влиянием УФ лучей является многократное здействие лучистой энергии в больших дозах, значительно превосхо- ящих дозы, применяемые с лечебной и профилактической целью.

Существующие данные литературы свидетельствуют о том, что при вместном действии нескольких канцерогенных факторов наблюдается иление бластомогенного эффекта, сокращение сроков малигнизации кани, повышение частоты появления опухолей или степени их злокаче- ственности [3, 6, 7, 15, 16, 19—21, 29, 30, 32]. Имеются указания, что нтакт организма с неактивными дозами канцерогена не проходит бесследно, а усиливает восприимчивость организма к последующему нцерогенному воздействию [8, 9].

Задача настоящего исследования заключается в том, чтобы уста- вить, изменяется ли бластомогенная активность УФ лучей у живот- ых, предварительно подвергнутых длительному хроническому воздей- ствию малых доз некоторых видов излучений (гамма-лучей, УФ лучей совместному действию гамма и УФ лучей), самостоятельно не способ- ных вызвать развития новообразований.

Table 2. ALBUMIN, GLOBULIN AND γ-GLOBULIN EXPRESSED AS PERCENTAGE TOTAL PROTEIN (T.P.) IN NORMAL AND HYPOPHYSECTOMIZED BABOONS

Baboon No.	Treatment	Albumin (% of T.P.)	Globulin (% of T.P.)	γ-Globulin (% of T.P.)
10 Controls	Nil*	57·48	42·52	—
26 Controls	Nil†	—	—	18·30
B 390	Normal*	56·50	43·50	—
	Hypophysectomized*	19·23	80·77	—
	Hypophysectomized†	18·30	81·70	50·30
B 413	Normal*	58·20	41·80	—
	Hypophysectomized*	36·40	63·60	—
	Hypophysectomized†	41·50	58·50	26·80
B 450	Normal*	57·30	42·70	—
	Hypophysectomized*	33·25	66·75	—
	Hypophysectomized†	30·71	69·29	33·10

* Biuret.
† Electrophoresis.

per cent) were significantly lower and the globulin levels (3·36–4·68 gm. per cent) higher than normal. The A/G ratio (1·378 in normal baboons) was markedly depressed to values between 0·278 and 0·571 (Table 1). Furthermore, γ-globulin concentrations in the operated animals, seven or more weeks after hypophysectomy, were significantly elevated above the normal level of 18·3 per cent of total protein[2]. γ-Globulin concentration in *B*413 had risen to 26·8 per cent of the total protein, to 33·10 per cent in *B*450 and to 50·3 per cent in *B*390 (Table 2).

*B*413 received 20 mgm. hydrocortisone daily for seven weeks. Both the total protein and albumin concentrations rose to levels well within the normal range (6·50 and 3·40 gm. per cent respectively). Weekly estimations of serum proteins, furthermore, showed the globulin concentration to have decreased by 0·48 gm. per cent to a level of 3·10 gm. per cent, which may be considered normal. The increasing albumin and decreasing globulin concentrations during the period of hormone treatment are thus reflected in the changing A/G ratio, which rose from 0·622 to 1·098 (Table 3).

Table 3. EFFECT OF HYDROCORTISONE THERAPY ON HYPOPHYSECTOMIZED BABOON, *B* 413

Treatment	Total protein (gm. %)	Albumin (gm. %)	Globulin (gm. %)	Albumin/Globulin
Nil	5·80	2·22	3·58	0·622
Hydrocortisone 20 mgm./day				
After 2 weeks	6·04	2·64	3·40	0·741
After 4 weeks	6·26	2·84	3·42	0·829
After 5 weeks and 2 days	6·38	3·28	3·10	1·058
After 6 weeks and 2 days	6·42	3·44	2·98	1·149
After 7 weeks and 2 days	6·50	3·40	3·10	1·098

Long-standing hypophysectomy in three baboons caused a reversal of the albumin/globulin ratio. This change is reflected both by a decrease of the serum albumin and an increase of the serum globulin concentration, which in large measure is a reflexion of an increase in γ-globulin, while the total serum protein-level was slightly depressed.

Levin and Leathem[3] concluded from their work on both adrenalectomized and hypophysectomized rats that alterations in the serum protein pattern following hypophysectomy could be attributed to an adrenal as well as a thyroid effect. They suggested that in the rat the serum albumin-level is maintained under adrenocortical influence while the serum globulin-level is associated with activity of the thyroid gland. Following hypophysectomy a change in both fractions occurs because of the decrease of both adrenocortical and thyroid activity.

Hartman *et al.*[4] demonstrated a drop in serum albumin and a rise in serum globulin following adrenalectomy in rats. In one of our hypophysectomized baboons, the elevated serum globulin was reduced to within the normal range after treatment with hydrocortisone. Indeed, the albumin concentration was more markedly affected than the globulin by hydrocortisone therapy. Albumin concentration rose by nearly 1·2 gm. per 100 ml. serum but the globulin-level was decreased by 480 mgm. per 100 ml. serum.

In view of the observations in baboons cited here, the reversed albumin/globulin ratio in kwashiorkor, like the fatty liver, is most probably dependent on a particular endocrine physiology which emerges in malnutrition and in kwashiorkor.

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β-Hydroxybutyrate and Sodium Citrate as Stimuli of the *in vitro* Secretion of Insulin

BRACHMACHARI *et al.*[1,2] have shown that certain metabolites like β-hydroxybutyrate, citrate, isocitrate and succinate, when injected into the animal (rabbit), produce hypoglycæmia, and this is interpreted as being due to a stimulus of the secretion of insulin. β-Hydroxybutyrate and citrate, according to the aforementioned experiments, seem to act, directly or indirectly, by different mechanisms, in view of the fact that the effect obtained is considerably more with citrate than with β-hydroxybutyrate and because this metabolite seems to increase first the content of active insulin/gm. of pancreas.

As we have shown that pancreas slices when incubated *in vitro* respond to certain stimuli (with insulin secretion) we thought it would be interesting to find out if β-hydroxybutyrate and citrate, when added to the buffer surrounding pancreas slices, produce an increase in secretion of insulin.

Table 1. INSULIN EFFECT OF THE INCUBATION BUFFER PLUS β-HYDROXYBUTYRATE OR SODIUM CITRATE SURROUNDING PANCREAS SLICES (No. of experiments in parentheses)

Metabolites	Glucose uptake (mean)		
β-Hydroxybutyrate 4×10^{-4} *M* (30)	0·470	± 0·017	$P > 0·05$
Control	0·436		
Sodium citrate 1×10^{-3} *M* (20)	0·574	± 0·029	$P < 0·01$
Control	0·388		
Sodium citrate 7×10^{-5} *M* (30)	0·331	± 0·013	$P = 0·01$
Control	0·281		

The technique already described[3] was adopted, using concentrations of citrate of 1×10^{-3} and 7×10^{-5} *M* and 4×10^{-4} *M* of β-hydroxybutyrate. As can be seen in Table 1, the citrate causes secretion of insulin, but not β-hydroxybutyrate, at the given dosage. This fact suggests to us that if both metabolites cause secretion of insulin in the animal, it does so by different mechanisms. Possibly by an indirect way in the case of the β-hydroxybutyrate and by a direct mechanism in the

case of the citrate. It could be by synthesis of adenosine triphosphate.

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Correlation of the Effects of Insulin and of Muscular Contraction *in vitro* on Uptake of Glucose by the Frog

Heimreich and Cori[1] and later R-Candela[2] among others have shown that the specificity to sensitive sugars is similar both with insulin and muscular contraction. On the other hand, the great resistance of toads to insulin injections is known from the work of Rapela *et al.*[3]. We have confirmed this finding in frogs injected with up to 40 U./100 gm. without showing any signs of hypoglycæmia.

These results inspired us to examine the possible correlation of the effects of insulin *in vitro* and of muscular contraction *in vitro* on uptake of glucose by the frog recti abdominis muscle.

Frogs (*Rana esculenta* L.) weighing 28–35 gm. were used. After killing, the muscles were removed and weighed; one of the muscles was always used as control of the other. They were incubated in small vessels in a Dubnoff shaker at 37° C. and stirred for 2 hr. The incubation medium was 2 ml. of frog-Ringer containing 3 mgm./ml. glucose. Contraction was elicited by incorporation of 10^{-5} *M* acetylcoline. Insulin was used at a concentration of 0·1 and 0·5 mU./ml.

In another series of experiments alternating 15-min. periods of contraction and relaxation were induced in the same muscle, by transferring it from the buffer containing acetylcholine to the relaxation buffer and vice versa, after very careful washing and blotting on filter paper.

Determinations of glucose were carried out by the Somogyi–Nelso method.

The results shown in Table 1 are different from those described for mammals:

(*a*) Gemmill *et al.*[4,5] showed that uptake of glucose and synthesis of glycogen are stimulated in the rat diaphragm *in vitro* by the addition of insulin. Our results, on the other hand, show that insulin does not increase uptake of glucose by frog muscle.

(*b*) Helmreich[1] and R-Candela[2] have shown, using different experimental conditions, that muscular contraction increases the uptake of glucose sugars with a specificity similar to that found when using insulin.

Table 1

	Without insulin	With insulin (0·5 U./ml.)	Alternative contraction and relaxation without insulin
Contraction	0·171 ± 0·041 (20)	0·180 ± 0·047 (20)	Contraction every 15 min. 0·158 ± 0·036 (20)
Relaxation	0·170 ± 0·041 (20)	0·201 ± 0·047 (20)	Relaxation every 15 min. 0·148 ± 0·036 (20)

No significant differences.

In the case of the frog, muscular contraction does not increase uptake of glucose.

Though Gemmill[6] and Beloff-Chain[7] state that insulin does not increase oxygen consumption by mammalian muscles, Hall[8], Gourley[9], Fisher[10], Smillie and Manery[11] find that insulin increases significantly the consumption of oxygen by frog skeletal muscle.

These experimental findings indicate the existence of a correlation between the effects of insulin and muscular contraction on uptake of glucose by muscle.

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Reversible Blocking of Nerve Conduction by Alternating-Current Excitation

IN recent years, the attention of control engineers has been attracted to the communication characteristics of nerves, particularly in regard to their function in the feedback control of muscle. Muscles are highly non-linear power amplifiers, which require complex nervous control circuitry to permit smooth and continuous movement of the limbs. This control circuitry consists of multiple feedback loops, and the problem is to determine experimentally the function of individual loops. One approach is to open circuit specific peripheral neural paths and observe the effect on over-all performance.

This can be done, and problems encountered with surgical and pharmacological methods avoided, by subjecting the nerve to alternating-current excitation. This method offers the advantage of reversibility, and also enables fibres to be selectively blocked according to their size. An investigation has been undertaken on peripheral nerves to study the effect of alternating-current excitation on axonal conduction. The sciatic nerves of large green frogs were used and a section of nerve, approximately 8 cm. in length, extending from the thigh to the peroneal and tibial extensions, was supported on an array of six platinum electrodes in a bath of mineral oil (see Fig. 1*a*). A pulse was applied repetitively to the stimulating electrodes and the response at the recording electrodes was amplified and displayed on an oscilloscope. The centrally located blocking electrodes were excited from an audio oscillator. Since the signal at the recording electrodes contained, superimposed, both the time-response of the nerve and an alternating-current component due to the ohmic coupling with the oscillator, a subtraction circuit was incorporated

In Vitro Measurements of the Effects of Anticoagulants on the Flow Properties of Blood: The Relationship of these Effects to Red Cell Shrinkage

By William I. Rosenblum

THE APPARENT RELATIVE VISCOSITY (ARV) of blood usually decreases as the hematocrit decreases.[1-4] However, the data described in the following report indicate that the ARV will increase if hematocrit is reduced by exposure of blood to anticoagulants, like citrate, which induce erythrocyte shrinkage.[5] A similar phenomenon has been reported by other workers,[6] but appears to have attracted little notice.

Material and Methods

Animals

Blood was obtained by cardiac puncture from anesthetized male mice, and by venipuncture from unanesthetized rabbits and monkeys.

Anticoagulation

Mouse blood was drawn into syringes containing 0.02 ml. of anticoagulant. Rabbit and monkey bloods were ejected from syringes into clean tubes containing dry sodium citrate, sodium oxalate or disodium ethylene diamine tetracetic acid (EDTA), or containing an aqueous solution of heparin (final concentrations of all anticoagulants are indicated in the text). When ACD solution (NIH formula B) was used, each ml. of blood was diluted with 0.25 ml. of ACD so that it contained 9 mg. sodium citrate, 3 mg. citric acid and 9 mg. dextrose. For comparison with ACD-blood, 4 parts of heparinized blood (1 U heparin per ml. blood) were diluted with 1 part normal saline. In three additional experiments, controls were prepared by adding 9 mg. dextrose to each ml. of diluted heparinized blood.

Measurements

Microhematocrit determinations were made on all samples. The apparent relative viscosity (ARV) was measured in a capillary viscosimeter of the type designed by Swank and Roth.[7] This instrument resembles, in principle, the viscosimeters used by Fahreus and Lindquist[8] and others.[9] Blood was drawn by a negative pressure of −100 mm. Hg. into a horizontal steel capillary 36 mm. long and approximately 306μ in diameter. A hand activated electric stopwatch, accurate to 0.01 seconds, was used to measure the time taken by 0.1 ml. of blood to pass through the capillary into a calibrated reservoir. The blood and apparatus were kept at 38 C. The time taken by blood was compared with that taken by an

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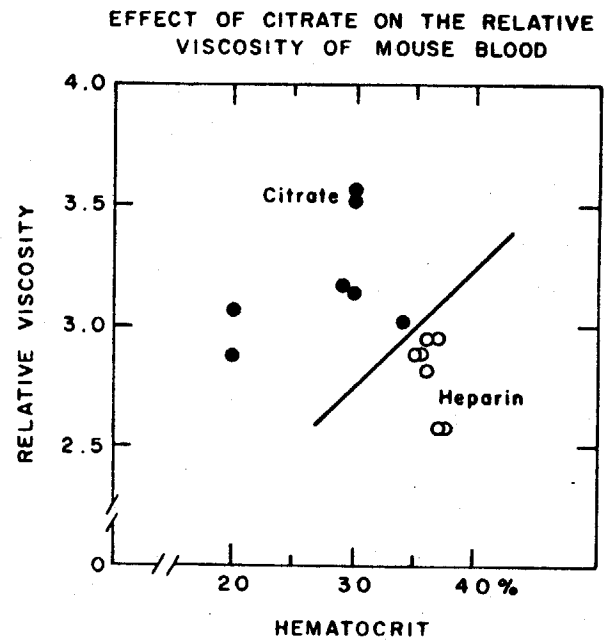

Fig. 1.—Heparin dosage was 2–4 U per ml. The concentration of sodium citrate was 5–10 mg per ml. The relative viscosity was higher in 6 of the 7 citrated bloods than in any of the controls, while the hematocrits were lower.

equal volume of distilled water under the same conditions (0.1 ml. water took 1 sec. to pass through the capillary in these experiments).

$$\text{ARV} = \frac{\text{Time taken by blood}}{\text{Time taken by water}}$$

In some experiments the viscosity index was also determined.[8]

$$\text{Viscosity index} = \frac{\text{ARV} \times 10}{\text{Hematocrit}}$$

This parameter enables one to more easily compare the viscosities of two samples with widely disparate hematocrits by providing a correction for the effect of hematocrit itself. This is made possible by two facts: (a) the relationship of ARV to hematocrit is essentially linear between hematocrits of 30 and 50[1-4] and (b) the slope of the line representing this portion of the relationship is relatively constant.

Results

Effects of Citrate

Figures 1 and 2 summarize the results obtained when citrated and heparinized mouse bloods were compared. Citrate reduced the hematocrit but elevated

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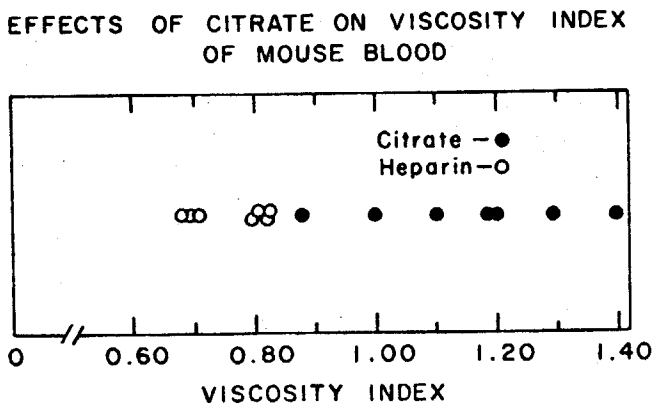

Fig. 2.—The data from figure 1 is replotted to show viscosity indices in the 2 groups of animals. All the citrated samples have viscosity indices greater than any of the heparinized samples. Viscosity index $= \frac{\text{relative viscosity}}{\text{hematocrit}} \times 10$.

the ARV and viscosity index. The differences between citrated and heparinized samples with respect to the distribution of values representing ARV, viscosity index and hematocrit are each significant at the .002 level (Mann-Whitney Test[10]). When a citrated aliquot from a given rabbit, monkey or mouse was compared with a heparinized aliquot from the same animal, the hematocrit was reduced and the ARV elevated (Figure 3, p = .002 Sign Test). Citrate had no effect on the viscosity of plasma.

Effects of Citrate on Heparinized Cells

Five mg. of Na Citrate were added to each ml. of pooled, heparinized mouse blood. Two experiments were performed. In both, the addition of citrate elevated the ARV and viscosity index (Table 1).

Effects of Citrate in ACD Solution

Monkey blood was diluted with ACD solution (Methods) and compared with heparinized (1U/ml.) blood from the same animal to which saline or saline plus dextrose (Methods) had been added. In only one out of six experiments (Table 2) was there a significant elevation in both the ARV and viscosity index, while in four experiments, a slight to moderate fall in these parameters was noted.

Effects of Sodium Oxalate and EDTA

Dry sodium oxalate,[11-13] like citrate, will shrink erythrocytes when added to blood. Oxalated blood (5 mg Na oxalate per ml.) displayed an increased ARV and viscosity index in 5 out of 6 animals when compared with heparinized (1U heparin per ml. blood) blood from the same animal (Table 3). Additional studies revealed that oxalate had no effect on the ARV of plasma. EDTA, like

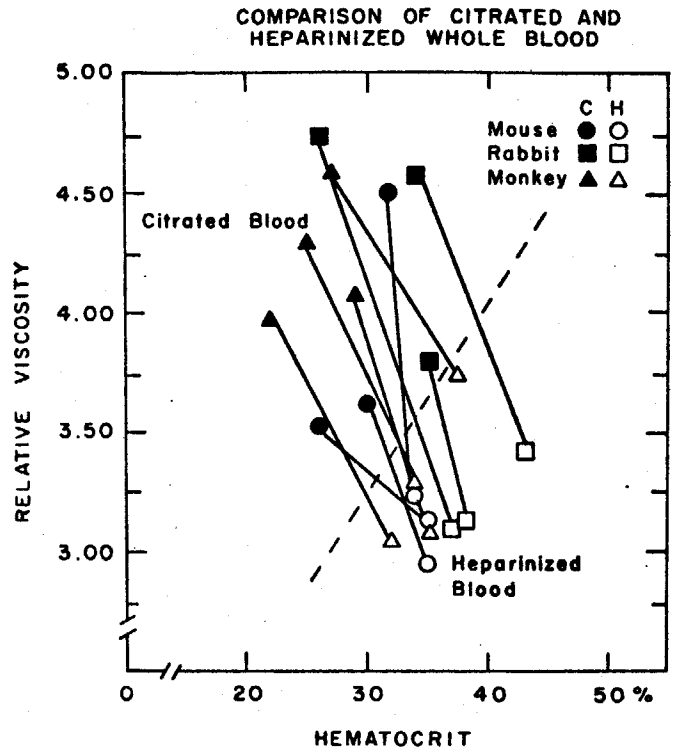

Fig. 3—The lines in the figure connect the 2 points representing samples from a single animal. Citrate elevated the relative viscosity and decreased the hematocrit. Citrate dosage was 3–5 per mg per ml. Heparin dosage was 1 U per ml. in monkey and rabbit blood and 4–5 U per ml. in mouse blood.

citrate and oxalate, removes calcium from the blood, but unlike the latter anticoagulants, EDTA leaves cell size and shape intact.[14] EDTA (1 mg./ml.) failed to significantly elevate ARV or viscosity index (Table 3).

Effects of Cell Shrinkage Induced by NaCl

Heparinized rabbit blood was centrifuged and the erythrocytes resuspended in phosphate-buffered NaCl solutions of increasing tonicity. As the concentration of NaCl increased and the cells shrank, there was a progressive rise in the viscosity index (Fig. 4).

Discussion

The anticoagulants used in this study can be divided into two groups: (a) those which shrink erythrocytes—citrate[1,5,6] and oxalate,[11-13] and (b) those

Table 1.—Effects of Na Citrate on Heparinized Pooled Mouse Blood

	Experiment 1		Experiment 2	
	*Heparin alone	*Heparin plus †citrate	*Heparin alone	*Heparin plus †citrate
Hematocrit	40	35	39	32
Relative Viscosity	2.940	3.100	3.360	3.670
Viscosity Index	0.740	0.890	0.740	1.130

*Heparin 4–5 U/ml blood.
†Citrate 5 mg/ml of heparinized blood.

Table 2.—Effects of ACD Solution on the Relative Viscosity and Viscosity Index of Monkey Blood

Animal Number	Heparin	ACD	Heparin + Dextrose
1	3.760* (0.990)†	3.230 (0.900)	———
2	3.970 (1.070)	3.140 (0.920)	———
3	4.590 (1.020)	3.250 (0.810)	———
4	———	3.100 (1.010)	2.920 (0.970)
5	———	3.900 (1.340)	2.760 (1.000)
6	———	3.070 (0.850)	3.280 (0.910)

*Apparent relative viscosity.
†Viscosity index.

Table 3.—Effects of Oxalate and EDTA on Blood Viscosity

Species	Age	Treatment	Relative Viscosity	Viscosity Index
Rabbit	6 mo.	Heparin	3.200	0.800
		Oxalate	3.970	1.140
Rabbit	26 mo.	Heparin	3.070	0.780
		Oxalate	4.380	1.330
Rabbit	36 mo.	Heparin	3.430	0.840
		Oxalate	4.210	1.080
Monkey (*M. irus*)	Adult	Heparin	3.760	0.990
		Oxalate	4.120	1.210
		EDTA	3.790	1.000
Monkey (*M. speciosa*)	Adult	Heparin	3.970	1.070
		Oxalate	4.720	1.450
		EDTA	3.870	1.050
Monkey (*M. speciosa*)	Adult	Heparin	4.590	1.020
		Oxalate	4.230	0.810
		EDTA	4.020	0.910

having no effect on cell size or shape—heparin,[11-13] EDTA[14] and ACD.[15,16] It is important to note that citrate is the anticoagulant agent in ACD solution, but red cell shrinkage is prevented when erythrocytes are exposed to citrate in this milieu. Heparinized blood was employed as the standard because heparin, in the dosages used here, does not alter blood viscosity.[17-19] Citrate or oxalate elevated the apparent relative viscosity (ARV) and viscosity index

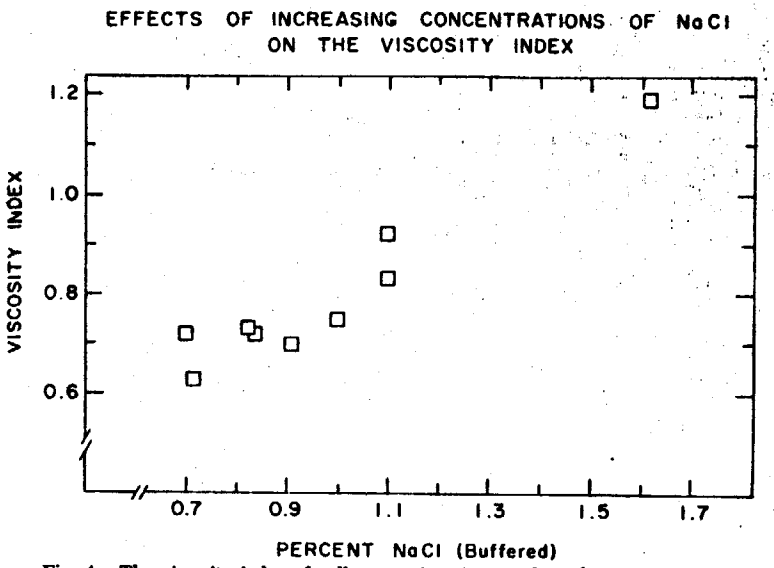

Fig. 4.—The viscosity index of cell suspensions increased as the tonicity of the suspending medium increased. The hematocrit was 30 in the suspension with the highest viscosity and 43 in the suspension with the lowest viscosity.

of whole blood, but had no effect on plasma viscosity, while EDTA or ACD failed to increase ARV or viscosity index. The data suggests that elevations in ARV were initiated by red cell shrinkage. This view is supported by the results of the experiments in which viscosity rose when heparinized cells were shrunk by exposing them to increasing concentrations of NaCl.

Data in the literature, pertinent to the present investigation, are sparse. Observations during the current study indicated that blood flowed 3 to 4 times more slowly than water, depending upon the species. Others have obtained similar results with dog blood and human blood, examined in viscosimeters with dimensions comparable to those employed herein.[7,9] Present observations on the effects of citrate confirm those of other workers utilizing a capillary viscosimeter of different dimension,[6] while the observations concerning oxalate and EDTA confirm the results of studies in which an entirely different type of viscosimeter was employed.[19,18] It would appear that the present data were not the product of a particular technic of measurement. This impression is further supported by the rare reports relating blood viscosity to erythrocyte size and to plasma tonicity. In the latter investigations, viscosimeters were used which differed greatly in design or dimenisons from the instrument employed in the present study. Trevan[1] observed an inverse relationship between blood viscosity and the size of erythrocytes altered by manipulating the osmotic pressure of their environment, while an investigation published

shortly after preparation of the present report demonstrates a striking relationship between plasma tonicity and blood viscosity.[20] The latter results support our conclusion that citrate and oxalate increased blood viscosity because these anticoagulants produced erythrocyte shrinkage.

The reason for an association between erythrocyte shrinkage and increased blood viscosity are not clear. Increased aggregation of red blood cells results in increased blood viscosity[21,22] and may be caused by alterations of the cell surface.[23] The cell membrane is certainly altered by erythrocyte shrinkage and these changes may result in cellular aggregation; however, the high shear rate at which the present observations were made would tend to disrupt aggregates.[21,22] Consequently, it may be that some other mechanism, such as increased cellular rigidity,[20,21] is responsible for the altered flow properties of shrunken cells.

Summary

Citrate and oxalate increased the viscosity of blood as measured in a capillary viscosimeter. The elevated viscosity was accompanied by erythrocyte shrinkage, manifest by the decreased hematocrit of blood anticoagulated with either of these agents. Plasma viscosity was not affected. EDTA, which does not alter cell size or shape, also failed to alter blood viscosity, while citrate no longer affected viscosity, if utilized in ACD solution, a milieu which prevents cell shrinkage. When erythrocytes were suspended in hypertonic NaCl, "blood" viscosity was also elevated in comparison to that of suspensions in lower concentrations of NaCl. The data indicate that blood viscosity will be elevated by anticoagulants which are permitted to shrink erythrocytes.

SUMMARIO IN INTERLINGUA

Citrato e oxalato augmentava le viscositate de sanguine secundo mesurationes in un viscosimetro capillari. Le elevate viscositate esseva accompagniate per retraction erythrocytic, manifeste in le deprimite hematocrite de sanguine tractate con le un o le altere del mentionate agentes como anticoagulantes. Le viscositate del plasma non esseva afficite. EDTA, que non altera le dimensiones o le conformation del cellulas, etiam esseva sin influentia super le viscositate del sanguine, durante que citrato cessava afficer le viscositate quando illo esseva utilisate in un solution in ACD (le qual preveni le retraction cellular). Quando erythrocytos esseva suspendite in hypertonic solution salin, le viscositate del "sanguine" esseva etiam augmentate in comparation con illo de suspensiones in plus basse concentrationes de NaCl. Le datos indica que le viscositate del sanguine es augmentate per anticoagulantes que resulta in un retraction del erythrocytos.

ACKNOWLEDGMENTS

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The Effect of Hyper-and Hypotonic Solutions and of Sodium Citrate Solutions on the Carotid Sinus

By Paolo Rowinski

The perfusion of an isolated organ with hyper-and hypotonic solutions causes in the first case vasodilatation, in the second vasoconstriction (Rowinski, 1). It is further known that hypertonic solutions injected intravenously cause a lowering of the pressure which may be the result either of peripheral vasodilatation, or of a reflex starting from the sensitive zones of the vessels, represented for example by the glomus carotideum or by other homologous zones. Margaria and Martini (2) were able to observe that this lowering of the pressure occurs also after enervation of the carotid sinuses, which, however, does not rule out that the reflex can start from other parts of the vascular system. I wanted to see, therefore, if it was possible to stimulate the carotid sinus with hyper-and hypotonic solutions or with isotonic solutions of various composition. All solutions were brought to pH 7.4 by addition of sodium bicarbonate. The solutions were either injected into the vascularly isolated sinus, through the upper thyroidal, or the sinus was perfused at constant pressure across the upper thyroidal and the lingual, while all other ramifications of the carotid were tied. The blood circulation in the sinus could be restored by opening the common carotid and the external maxillary, which at the time of the injection or perfusion had been provisionally closed. The temperature of the liquids was maintained at 37°. The cryoscopic lowering of the isotonic solutions was -0.56°. The action on the sinus was observed by recording the pressure with a mercury gauge in the femoral and by recording the breathing with Benedict's spirometer.

Iso-osmotic solutions of glucose, urea, and sodium chloride have no effect on the carotid sinus. Sodium sulfate causes sometimes a slowing of the breathing, but this effect is irregular and uncertain.

Very hypertonic solutions of sodium chloride and also of glucose cause, instead, an increase of the frequency and amplitude of the breathing and at the same time an increase in blood pressure which, in the case of sodium chloride, is followed by a reduction. After enervation of the sinus, the same solutions are without effect. The same solution of sodium chloride injected intravenously causes variable effects on the breathing and a considerable lowering of the pressure only in much higher quantities.

The injection of 1 cc of the iso-osmotic solution of sodium citrate into the carotid sinus causes consistently a stoppage of the breathing and a lowering of the blood pressure. After enervation of the barosensitive zone situated at the internal carotid, while the innervation of the glomus carotideum remains intact, the injection causes instead an increase of the pressure while inducing a stoppage of the respiration. The same occurs after cutting of the vagus nerves.

Injection of 3 cc of the same citrate solution toward the centers through the internal carotid has a much more modest effect on respiration and pressure. In any case, the pressure rises and the breathing stops.

Total enervation of the sinus abolishes the reaction.

According to von Euler (3), the potassium salts have a stimulating action at low concentration, a paralyzing action at higher concentration. In fact, he notes b injection into the sinus of very small quantities (50 gamma) an increase of the respiratory frequency and an increase of the arterial pressure. Injection toward the centers, instead, causes lowering of the pressure and stoppage of the respiration, and the effect manifests itself probably, according to v. Euler, independently of a cardiac action. Hauss and Shen (4) find a reduction of the pressure and a stoppage of the respiration preceded by an increase of the breathing under the action of the potassium salts in the carotid sinus. According to Mathison (5), the potassium salts injected intravenously cause a lowering of the pressure through their activity on the heart. The intra-arterial injection, however, owing to which the potassium salts do not get to the heart directly, causes an increase of the pressure

due either to the stimulation of the vasomotor centers, or to the direct stimulation of the vessel wall. I have been able to note sometimes by injection of sodium citrate the action of the small doses of potassium salts observed by Euler, and nearly always that of the large doses found also by Hauss and Shen and which consists of a stoppage of the respiration with lowering of the pressure. But the action of the potassium salts on the sinus varies also according as their action is exerted either on the whole sinus or on the sinus after enervation of the baroceptors. In the latter case, instead of a reduction of the pressure, an increase occurs, and the same increase is observed when the solution is injected directly toward the centers through the internal carotid, contrary to what v. Euler had seen.

It is probable, therefore that sodium citrate (absence of $Ca^{\cdot\cdot}$) stimulates in the sinus either the baroceptors or the chemoceptors. At high doses the action of the baroceptors on the pressure prevails, the stimulation of which causes a lowering of the pressure, while on the respiration the stimulating action would, in most cases, when the chemoceptors are not enervated, manifest itself with the stoppage of breathing comparable to the stimulation of the central stump of the vagus; sometimes I was able to note also an increase of the frequency and amplitude of the breathing, which may perhaps be explained by the fact that the citrate solution does not reach the glomus carotideum in sufficient concentration.

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(1) Atti Soc. It. Progr. Sc., 1939 (in course of publication)

(2) This Journal, 1940, 15, 225.

(3) Skand. Arch. f. Physiol., 1938, 80, 94.

(4) Arch. Int. Pharmakodyn., 1939, 62, 411.

(5) J. Physiol., 1911, 42, 471.

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sviluppo di sistema nervoso (6). Queste esperienze, combinate con quelle del paragrafo 1, dimostrano che NaSCN agisce esaltando i processi che presiedono alla determinazione dell'abbozzo cordale e liberano la sostanza evocatrice che fa formare il sistema nervoso a spese dell'ectoderma banale (7). E' noto che ambedue questi processi sono legati al metabolismo idrocarbonato, per cui il meccanismo di azione di NaSCN potrebbe essere nei due casi lo stesso.

(6) Ranzi S. e Tamini E. - *Naturwiss.*, 1940, 28, 458; *Rend. R. Ist. Lombardo Sc. e Lett.* (in corso di stampa).
(7) Per maggiori chiarimenti bibliografici cfr. Ranzi S. - *Chimica e Industria*, 1940, 22, 217.

(Dall'Istituto di Zoologia e Anatomia comparata della R. Univ. di Milano).

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ANEMIA MACROCITICA IPERCROMICA SPERIMENTALE E PRINCIPIO ANTIANEMICO. - III. Di A. ROSSI e R. CONTE-MAROTTA.

In precedenti ricerche (1) abbiamo dimostrato che, per azione del succo gastrico umano incubato con lievito autolizzato, si forma una sostanza termostabile, solubile in alcool al 70 % ed insolubile in alcool al 90 %, che, somministrata parenteralmente in conigli resi anemici mediante trattamento con saponina e collargolo, provoca intensa reazione reticolocitaria, aumento degli eritrociti e guarisce l'anemia sperimentale. La stessa sostanza, somministrata per via parenterale in due casi di anemia perniciosa, ha dato risultati soddisfacenti. In questa nota riferiamo su esperienze eseguite allo scopo di indagare quale dei costituenti del lievito può funzionare da fattore estrinseco. Abbiamo, per ora, preso in esame l'acido nicotinico, la lattoflavina e la così detta « frazione del filtrato ».

Le esperienze sono condotte nel seguente modo: 25-30 cc di succo gastrico umano normale, prelevato a digiuno dopo iniezione di istamina, e filtrato per garza, si mescolano con circa 5 cc di una soluzione contenente mg 0,5 di lattoflavina (sintetica, Bayer) e mg 9 di acido nicotinico purissimo, o con circa 30 cc della « frazione del filtrato » dal lievito preparata secondo Edgar e Macrae e concentrata a piccolo volume nel vuoto. Si porta a pH 5 e si lascia in termostato a 37° per 6 ore agitando spesso. Dopo tale periodo di tempo si riscalda a 65° per 30 min. per inattivare la emopoietina, si filtra e il filtrato si addiziona con alcool sino alla concentrazione del 70 %. Si forma un precipitato che si separa per filtrazione; il filtrato si concentra a piccolo volume nel vuoto e a bassa temperatura. Si aggiunge alcool assoluto sino alla concentrazione del 90 %. Si forma un precipitato che si separa per centrifugazione. Si è saggiata l'attività sia del precipitato disciolto in poca acqua, sia del liquido concentrato a piccolo volume.

I risultati di queste esperienze mostrano che, mentre l'acido nicotinico e

(1) *Rend. R. Accad. Italia*, 1940, 1, 295; *Questo Bollettino*, 1940, 15, 543.

la lattoflavina, incubati con succo gastrico, non hanno alcun effetto, la somministrazione del preparato, ottenuto incubando la « frazione del filtrato, produce un rapido e profondo miglioramento del quadro ematico con aumento degli eritrociti (di 2.500.000 in media), crisi reticolocitaria (da 1 % a 8 %) e ripristino del normale valore globulare.

I risultati di queste ricerche dimostrano che la sostanza del lievito, che incubata con succo gastrico forma il principio antianemico che guarisce la anemia ipercromica macrocitica dei conigli, si trova nella così detta « frazione del filtrato ».

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L'EFFETTO DI SOLUZIONI IPER- E IPOTONICHE E DELLE SOLUZIONI DI CITRATO SODICO SUL SENO CAROTIDEO. Di PAOLO ROWINSKI.

La perfusione di un organo isolato con soluzioni iper- ed ipotoniche determina nel primo caso una vasodilatazione, nel secondo una vasocostrizione (Rowinski, 1). È noto inoltre che soluzioni ipertoniche iniettate per via endovenosa determinano un abbassamento della pressione che potrebbe essere la conseguenza sia della vasodilatazione periferica, sia di un riflesso con partenza dalle zone sensibili dei vasi rappresentato, ad es., dal glomo carotideo o da altre zone omologhe. Margaria e Martini (2) hanno potuto osservare che questo abbassamento della pressione si verifica anche dopo l'enervazione dei seni carotidei, il che però non esclude che il riflesso possa partire da altre parti del sistema vasale. Ho perciò voluto vedere se era possibile eccitare il seno carotideo con soluzioni iper- ed ipotoniche o con soluzioni isotoniche di varia composizione. Tutte le soluzioni erano state portate a pH 7,4 con l'aggiunta di bicarbonato sodico. Le soluzioni o venivano iniettate nel seno vascolarmente isolato, attraverso la tiroidea superiore, o si perfondeva il seno a pressione costante attraverso la tiroidea superiore e la linguale, mentre erano state legate tutte le altre ramificazioni della carotide. Il circolo sanguigno nel seno poteva essere ristabilito con l'apertura della carotide comune e della mascellare esterna, che al momento dell'iniezione o della perfusione venivano provvisoriamente chiuse. La temperatura dei liquidi era mantenuta a 37°. L'abbassamento crioscopico delle soluzioni isotoniche era di —0,56°. L'azione sul seno veniva studiata mediante registrazione della pressione con manometro a mercurio nella femorale e mediante registrazione del respiro con lo spirometro di Benedict.

Soluzioni isoosmotiche di glucoso, di urea e di cloruro sodico non hanno

(1) *Atti Soc. It. Progr. Sc.*, 1939 (in corso di pubbl.).
(2) *Questo Bollettino*, 1940, 15, 225.

effetto sul seno carotideo. Il solfato sodico determina talora un rallentamento del respiro, ma quest'effetto è incostante e incerto.

Soluzioni molto ipertoniche di cloruro sodico e anche di glucoso, determinano invece un aumento della frequenza e dell'ampiezza del respiro e contemporaneamente un aumento della pressione sanguigna che, nel caso del cloruro sodico, è seguito da una diminuzione. Dopo enervazione del seno, le stesse soluzioni sono senza effetto. La stessa soluzione di cloruro sodico iniettata per via endovenosa determina solo in quantità molto superiori effetti variabili sul respiro ed un abbassamento notevole della pressione.

L'iniezione di 1 cc della soluzione isoosmotica del citrato sodico nel seno carotideo determina costantemente un arresto del respiro ed un abbassamento della pressione sanguigna. Dopo enervazione della zona barosensibile situata in corrispondenza della carotide interna, rimanendo integra l'innervazione del glomo carotideo, l'iniezione determina invece un aumento della pressione, pur provocando un arresto del respiro. Lo stesso fatto si verifica anche dopo taglio dei vaghi.

L'iniezione verso i centri attraverso la carotide interna, di 3 cc della stessa soluzione di citrato, ha un effetto molto più modesto sul respiro e sulla pressione. In ogni modo si ha un aumento della pressione ed un arresto del respiro.

L'enervazione totale del seno abolisce la reazione.

Secondo von Euler (3) i sali potassici avrebbero a piccola concentrazione un'azione eccitante, a maggior concentrazione un'azione paralizzante. Difatti egli nota per iniezione nel seno di piccolissime quantità (50 γ) un aumento della frequenza respiratoria ed un aumento della pressione arteriosa. L'iniezione verso i centri invece determina abbassamento della pressione ed arresto del respiro, e l'effetto si manifesta probabilmente, secondo v. Euler, indipendentemente da un'azione cardiaca. Hauss e Shen (4) trovano invece una diminuzione della pressione ed un arresto della respirazione preceduto da un aumento del respiro per azione dei sali di potassio nel seno carotideo. Secondo Mathison (5) i sali di potassio iniettati per via endovenosa determinano un abbassamento della pressione per la loro attività sul cuore. L'iniezione endoarteriosa invece, per cui i sali di potassio non arrivano direttamente al cuore, determina un aumento della pressione dovuto sia all'eccitamento dei centri vasomotori, sia all'eccitamento diretto della parete vasale. Io ho potuto notare talora per iniezione di citrato sodico l'azione delle dosi deboli di sali di potassio osservata da Euler, sia quasi sempre quella delle dosi forti trovata anche da Hauss e Shen e che consiste in un arresto del respiro con abbassamento della pressione. Ma l'azione dei sali potassici sul seno si modifica anche a seconda che la loro azione si esplichi sia sul seno integro, sia sul seno dopo enervazione dei barocettori. Nel secondo caso, invece di una diminuzione della pressione si ha un aumento, e lo stesso aumento si osserva allorchè

(3) *Skand. Arch. f. Physiol.*, 1938, 80, 94.
(4) *Arch. Int. Pharmakodyn.*, 1939, 62, 411.
(5) *J. Physiol.*, 1911, 42, 471.

la soluzione viene iniettata direttamente verso i centri attraverso la carotide interna, contrariamente a quanto ha visto v. Euler.

È quindi probabile che il citrato sodico (assenza di Ca··) ecciti nel seno sia i barocettori sia i chemocettori. Con dosi forti, prevale sulla pressione l'azione dei barocettori, la cui stimolazione determina un abbassamento della pressione, mentre sul respiro l'azione eccitante si manifesterebbe nella maggioranza dei casi, allorchè i chemocettori non sono denervati, con l'arresto del respiro paragonabile alla stimolazione del moncone centrale del vago; talora ho potuto notare ancora un aumento della frequenza ed ampiezza del respiro che potrebbe forse spiegarsi con il potere che la soluzione di citrato non arriva al glomocarotideo in concentrazione sufficiente.

(Dall'Istituto di Fisiologia della R. Università di Torino).

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RESPIRO PERIODICO DI ORIGINE RIFLESSA. Di PAOLO ROWINSKI.

La respirazione periodica viene generalmente considerata come un fenomeno di origine centrale, legato ad una modificazione dell'eccitabilità del centro respiratorio, che sta in rapporto con oscillazioni periodiche dei gas del sangue. È stata anche prospettata la possibilità che il centro respiratorio possa venire eccitato e depresso per effetto di stimoli provenienti sia dai polmoni, sia dalla zona cardioaortica, sia dal seno carotideo. Gallerani (1) trova che la stimolazione del moncone del nervo depressore di Cyon modifica anche il respiro rallentando il ritmo e favorendo specialmente l'espirazione; in certe condizioni la stessa stimolazione determina l'insorgenza del respiro periodico, per cui il Gallerani pensa che stimoli della zona cardioaortica possano originare il respiro periodico.

Lavorando sul seno carotideo ho potuto notare come talvolta in animali che hanno una predisposizione al respiro periodico in seguito ad una narcosi troppo profonda ottenuta con l'iniezione endovenosa di Dial « Ciba », e nei quali erano stati legati tutti i rami della carotide comune ad eccezione della mascellare esterna, la chiusura della carotide comune o della mascellare o di ambedue determinava l'insorgenza di un respiro periodico passeggero. Poichè la chiusura della carotide comune determina in via riflessa un aumento della pressione ed un aumento della frequenza respiratoria, si potrebbe anche pensare che il respiro periodico si manifesti in seguito all'iperventilazione, con conseguente alterazione dei gas del sangue, ma questa non pare sia la causa, poichè anche la chiusura della mascellare esterna che porta ad una diminuzione della pressione generale e della frequenza respiratoria determina lo stesso fenomeno. Non mi è ancora possibile dire se il riflesso parte dalle terminazioni nervose baro- o chemosensibili del seno, poichè esso è stato finora

(1) *Arch. di Fisiol.*, 1914, 12, 1.

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Effect of Some Complexing Substances on the Elimination of Cesium-134 from the Organism

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The problem concerned with the elimination, from the organism, of radioactive substances incorporated in it, in particular, of radioactive cesium, is a very timely one.

Cesium belongs to a group of rare metals; only its stable isotope is encountered in nature. However, in the decomposition of uranium, there is formed a large amount of radioactive cesium. There exist 22 radioactive isotopes of cesium with varying half-lives -- ranging from tenth fractions of a second to 2.10^6 years. Upon finding their way into the organism, the radioactive substances complex with albumin and yield insoluble products. Only a small part of the radioactive substance can form soluble compounds that are readily eliminated from the organism.

The goal os papers (studies) on accelerating the elimination of radioactive substances is an increase in the formation of soluble complexes with these substances. By raising the concentration of organic acids in the liquids of the organism, it is possible to attain a shift in the reaction of forming soluble complex compounds in the direction that we require, and, at the same time, to increase the elimination of the radioactive substance from the organism.

In the literature that was accessible to us we did not encounter any indications pointing to the acceleration of eliminating radioactive cesium by means of complexing agents. The article of E. B. Kurlyandska has been devoted to the problem of accelerating the elimination of cesium-134 from the organism.

The author, by saturating the organism with a stable cesium isotope, was able to increase the elimination of cesium-134 in an amount altogether 10% greater than the one in the control group.

We made a study of the elimination of cesium-134 by means of complexing substances: oxalic acid, calcium gluconate, sodium citrate, and various combinations composed of these complexing agents.

Procedure Studies were carried out on 258 white rats with an average weight of 170 grams (plus or minus 10); 130 rats comprised the experimental group, and 128 -- the control. All the animals were administered 3 muC of radioactive cesium-134 in the abdominal cavity. After an hour, the experimental group was administered, additionally, one of the complexing substances that, subsequently, was administered once a day over an extent of five or ten days.

Oxalic acid was administered to one group of animals (dose -- 36 milligrams to a rat) in the abdominal cavity. The second group -- oxalic acid was mixed with water, which the rats drank up over a period of days. The daily dose, in this case, was equal to 50 milligrams of oxalic acid to one animal. Altogether, in five days, the rats of the first group received an amount of 180 milligrams of oxalic acid, and those of the second group -- an amount of 250 milligrams.

Sodium citrate was administered into the abdominal cavity and intramuscularly in a dose of 1 milliliter of a 10% solution to a rat. In five days, the animal received 5 milliliters of a 10% solution of sodium citrate. Solutions of sodium citrate and oxalic acid were prepared ex tempore on a physiological solution.

Calcium glucenate was administered to the rats in an amount of 1 milliliter of a 10% solution into the abdominal cavity. Sodium citrate (1 milliliter of a 10% solution) and oxalic acid (36 milligrams) -- simultaneously, likewise

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into the abdominal cavity. Calcium gluconate was administered into the abdominal cavity, at the same time, with sodium citrate.

The first group of animals was administered an amount of 0.5 milliliters of a 10% solution of calcium gluconate and 50 milligrams of sodium citrate. The second had its dose of calcium gluconate increased to 1 milliliter of a 10% solution; and that of the sodium citrate -- in an amount of 50 milligrams to a rat. All the animals were kept in exchange cages where, by means of a distributing funnel, urine and feces were collected from each rat individually.

Preparations were made from the selected material; a measurement was made of the daily amount of urine; 0.1 milliliter of the urine was deposited on a target and dried in a desiccator; the daily amount of feces was dried in a desiccator, and after tha, it was gound in a mortar and on the target was deposited a weighed portion of 50 milligrams. In order that the feces be distributed uniformly on the target, it was copiously wetted with ether; with the liquid it spread along the target. A count of the activity was carried out on a "B" unit with an end counter. Each preparation was counted four times for a period of two minutes, and after that, the arithmetic-mean indices were deduced. The activity of each preparation was recalculated for a daily amount of the material under study, and after that, a computation was made of the percents of daily elimination of cesium-134 with the urine and feces, relative to the dose that had been administered.

Oxalic acid, upon being complexed with the metals of the first group of the periodic system, yields readily soluble compounds. Cesium belongs to the first group of the periodic system and represents the most active metal. Consequently, it will also react with oxalic acid and form soluble complexes that are readily eliminated from the organism. However, upon administering oxalic acid to rats in the abdominal cavity, we did not observe any acceleration in its elimination.

In the experimental animals, at the places of administration of the oxalic acid, necroses were produced. The overall condition of the rats was poor. They did not take nourishment, and their furry coat was dirty and dishevelled. A day following the administration into the organism of cesium-134, in the test animals, with the experiments, there was administered a considerably smaller amount of cesium than in the controls. Over the extent of all the following days of observation, the amount of cesium eliminated in the urine and in the feces of the experimental rats was smaller than in the control.

In five days there was eliminated, in the experimental animals, on an average, with the urine and feces, 29.2% (with fluctuations from 25% to 30%), and in the control animals -- 31.3% of radioactive cesium (with fluctuations from 30 to 33%).

The appearance of necroses, in the case of intraperitoneal administration of oxalic acid, convinced us to change the method of administering it into the organism. In the following experiments, we began to mix the oxalic acid with sweet water, which the rats drank up over a period of days. No side effects were observed in this case. The behavior of the experimental animals in no way differed from the controls. The elimination of cesium-134 in the experimental animals, over a period of five days, accelerated by 23%. Thus, in the control group, there was eliminated, on an average, 29.8% of cesium-134, and in the experimental animals -- on an average, 52.9%, that is, half of the cesium-134 introduced into the organism was eliminated over a period of five days, whereas the period of semi-elimination of cesium-134 in the rats was equal, on an average, to 28 days.

Existing, numerous data in the literature, which are devoted to the effect of citric acid salts, in particular, sodium citrate, on the elimination of radioactive metals from the organism, do not pertain to the elimination of cesium-134 (Hackett, Shubert, Ketty). At the same time, cesium, as a more

active metal, and as an analog of sodium and potassium in the organism, can expel sodium from the compounds, while occupying its place. A complex substance -- cesium citrate -- is readily soluble, and for this reason, it can easily be eliminated from the organism.

Upon administering sodium citrate into the abdominal cavity, no changes were observed at the place of injection. The bahavior of the experimental animals did not differ from that of the controls. In the first days, however, there was noted a considerable difference in eliminating cesium-134 from the experimental and control animals. If, in the control rats, there was eliminated 7% of cesium-134, then in the experimental ones -- it was 12% of the radioactive cesium. Subsequently, in a day, in the experimental animals, there was eliminated, on an average, 3% more radioactive cesium than in the controls.

In the course of 5 days, in the experimental animals, there is eliminated, with the urine and feces, on an average, 48.3% of radioactive cesium-134 (with fluctuations ranging from 46% to 50%), and in the controls -- 33.1% (with fluctuations from 31 to 35%). Consequently, in five days, the amount of cesium introduced into the experimental rats increased, on an average, by 15% in comparison with the control group.

Upon administering sodium citrate intramuscularly, infiltrates make their appearance at the places of injection, and by the end of the observation (the fifth day), the infiltrated tissues are subject to necrotic changes. In these days, the overall condition of the experimental rats is poor. They refuse nourishment, their furry coat is dirty and dishevelled. No acceleration in elimination is observed.

In the first days, in the control animals, there is eliminated a considerably greater amount of radioactive cesium than in the experimental ones. In five days, in the experimental animals there was eliminated, on an average,

34.2%, and in the control -- 51% of radioactive cesium-134.

An analogous picture is observed upon administering, into the abdominal cavity, sodium citrate and oxalic acid. Necroses are observed at the place of injection, and the elimination of cesium-134 is decelerated. In the control animals, in this case, there was eliminated, on an average, in five days, 31.3% of cesium-134, and in the experimental ones -- only 15%.

Calcium gluconate and a combination of sodium citrate with calcium gluconate, for accelerating the elimination of radioactive cesium-134, was employed by us for the first time. Depending on the capacity of calcium to diminish the permeability and increase the turger (turgescence) of the cells (V. I. Skvortsov, P. N. Kravkov, N. V. Vershinin), we made an attempt to exert an influence on the elimination of cesium by means of calcium gluconate. The latter has been selected for the reason that it does not produce such tissue irritation as calcium chloride. Even after a day following the beginning of the experiment, the amount of cesium eliminated with the urine and feces, in the experimental animals, exceeded, by two times, the amount of cesium eliminated in the group of control animals: thus, on an average, with the urine and feces, in the experimental animals, there was eliminated 22.3%, and in the controls -- only 12% of radioactive cesium-134.

Over a period of five days, with the urine and feces, in the experimental animals, there was eliminated, on an average, 55.3% of cesium-134 -- half of the amount introduced into the organism, whereas, in the control animals, it was only 35.3%. Consequently, in five days, in the experimental animals, there was eliminated, on an average, 20% more radioactive cesium than in the controls.

By increasing the dose of celcium gluconate introduced, it becomes possible to increase, also, the elimination of cesium-134. In this case, just as in the preceding experiment, in the first days, in the experimental animals, there is eliminated, on an average, twice as much radioactive cesium as in the

control. If, in the control animals, with the urine and feces, in the first days, on an average, there was eliminated 14% of cesium-134, then in the experimental ones -- it was 28.7%. In the course of five days, in the control animals, ther was eliminated 34% of radioactive cesium, and in the experimental ones -- 67% -- 23% more than in the control. Over a period of nine days, in the control animals, there was eliminated, on an average, 40%, and in the experimental ones, 80% of radioactive cesium-134. In comparing the effect of calcium gluconate on the elimination of cesium-134, we disclosed that calcium gluconate accelerates the elimination of cesium negligibly -- on an average, 5% more in comparison with the control. In exerts an influence, for the main part, on diminishing the permeability of the cells, and consequently, is instrumental in diminishing the penetration of the radioactive substance inside the cells. The less of the radioactive substance penetrates into the cells. The less of the radioactive substance penetrates into the cell, the fewer the complex, insoluble compounds, on its part, that are formed with the albumins (proteins). Sodium citrate, introduced against this background, binds large amounts of cesium-134 and is instrumental in increasing its elimination from the organism.

Conclusions

1. Oxalic acid, administered to animals with their feed, accelerates the elimination of cesium-134, on an average, by 23%.

2. Sodium citrate, administered into the abdominal cavity, accelerates the elimination of cesium-134, on an average, by 15% in comparison with the control.

3. A combination of sodium citrate with calcium gluconate accelerates the elimination of cesium-134 over a period of five days by 23%, and over a period of nine days -- 40% more than in the control.

4. Calcium gluconate, administered into the abdominal cavity, accelerates the elimination of cesium-134, on an average, by 5% in comparison with the control.

5. Necroses and inflammatory changes of the tissues retard (inhibit) the elimination of cesium-134 from the organism.

(Translated by Carl Demrick Associates,Inc/LB/t)

Citrate
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язвенной болезнью оказалась отрицательной (—) у 17, у которых наступило практическое выздоровление и клиническое улучшение. У одного больного лечение сном эффекта почти не дало, реакция крови на ацетилхолин осталась положительной, но менее выражена, чем до лечения сном. Активность холинэстеразы крови у него осталась повышенной. После лечения сном у всех больных активность холинэстеразы снизилась до нормы и колебалась в пределах от 1,6 до 2,3 ед.

Особое внимание нами было уделено исследованию функции печени у язвенных больных при лечении их сном. Мы исследовали в динамике некоторые функции печени (антитоксическую, протромбинообразовательную, сахарные кривые крови и тимоловую пробу) у 100 больных, страдающих язвенной болезнью, подвергшихся лечению сном в нашей клинике.

Под влиянием лечения длительным медикаментозным сном к десятому дню лечения обнаружено ухудшение функциональной способности печени: в большей степени ухудшалась антитоксическая функция ее, что, по-видимому, связано с токсическим воздействием на печень недостаточно обезвреженных снотворных препаратов. Эти данные согласуются с выводами С. С. Ременник (1953) и М. Я. Квиркелия (1953). К концу лечения сном функции печени у некоторых больных улучшались, а у части полностью восстанавливались в связи с нормализацией центральной нервной регуляции, улучшением трофической функции нервной системы и исключением вредного токсического воздействия на печень снотворных средств (другую половину курса лечения мы проводили при помощи условнорефлекторного сна). Отмечено, что у больных, у которых антитоксическая функция печени была значительно понижена до лечения сном (ниже 50%), наблюдалось еще большее угнетение ее под влиянием снотворных к концу лечения сном.

Показатели протромбинового индекса, тимоловой пробы и сахарных кривых крови под влиянием длительного медикаментозного сна к десятому дню лечения несколько ухудшились, а к концу терапии сном их показатели значительно улучшились.

Отдаленные результаты исследования функций печени указывали на дальнейшее их восстановление лишь у тех больных, у которых не наступало обострений после проведенного лечения сном. У тех больных, у которых обострения язвенной болезни повторялись, функциональная способность печени не восстанавливалась, а имела тенденцию к снижению.

Наилучшим способом лечения сном страдающих язвенной болезнью, по нашему мнению, является метод комбинированного применения малых доз нескольких снотворных и смена медикаментозного сна условнорефлекторным. Эффективность лечения сном зависит не только от методики терапии, но и от правильного дифференцированного подхода к каждому больному при назначении им этого метода лечения.

На основании наших наблюдений ближайшие результаты лечения сном больных язвенной болезнью можно считать хорошими, отдаленные — удовлетворительными.

ВЛИЯНИЕ НЕКОТОРЫХ КОМПЛЕКСООБРАЗУЮЩИХ ВЕЩЕСТВ НА ВЫВЕДЕНИЕ ЦЕЗИЯ-134 ИЗ ОРГАНИЗМА

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Вопрос о выведении из организма инкорпорированных радиоактивных веществ, в частности радиоактивного цезия, весьма актуален.

Цезий принадлежит к группе редких металлов, в природе встречается только стабильный его изотоп. Но при распаде урана образуется большое количество радиоактивного цезия. Существует 22 радиоактивных изотопа цезия с различными периодами полураспада — от десятых долей секунды до 2.10^6 лет. Попав в организм, радиоактивные вещества комплексируются с белком и дают нерастворимые продукты. Только небольшая часть радиоактивного вещества может образовывать растворимые соединения, которые легко выводятся из организма.

Целью работ по ускорению выведения радиоактивных веществ является увеличение образования растворимых комплексов с этими веществами. Повышая концентрацию органических кислот в жидкостях организма, можно добиться сдвига реакции образования растворимых комплексных соединений в нужную нам сторону и тем самым увеличить выведение радиоактивного вещества из организма.

В доступной нам литературе указаний на ускорение выведения радиоактивного цезия с помощью комплексообразователей мы не встретили. Вопросу ускорения выведения цезия-134 из организма посвящена статья Э. Б. Курляндской. Автор, насыщая организм стабильным изотопом цезия, смогла увеличить выведение цезия-134 всего на 10% больше, чем в контрольной группе.

Мы изучали выведение цезия-134 с помощью комплексообразующих веществ: щавелевой кислоты, глюконата кальция, лимоннокислого натрия и различными комбинациями, составленными из этих комплексообразователей.

Методика. Исследования проведены на 258 белых крысах со средним весом 170 г (±10), 130 крыс составили подопытную группу, 128 — контрольную. Всем животным вводили по 3 μС радиоактивного цезия-134 в брюшную полость. Через час подопытной группе вводили дополнительно одно из комплексообразующих веществ, которое впоследствии вводилось раз в сутки на протяжении пяти или десяти дней.

Щавелевую кислоту вводили одной группе животных (доза — 36 мг на крысу) в брюшную полость. Второй группе — щавелевая кислота подмешивалась к воде, которую крысы выпивали в течение суток. Суточная доза в этом случае равнялась 50 мг щавелевой кислоты на одно животное. Всего за пять дней крысы первой группы получили по 180 мг щавелевой кислоты, второй группы — по 250 мг.

Лимоннокислый натрий вводился в брюшную полость и внутримышечно в дозе 1 мл 10% раствора на крысу. За пять дней животное получало 5 мл 10% раствора лимоннокислого натрия. Растворы лимоннокислого натрия и щавелевой кислоты готовились ex tempore на физиологическом растворе.

Глюконат кальция вводился крысам по 1 мл 10% раствора в брюшную полость. Лимоннокислый натрий (1 мл 10% раствора) и щавелевая кислота (36 мг) — одновременно также в брюшную полость. Глюконат кальция вводился в брюшную полость одновременно с лимоннокислым натрием.

Первой группе животных вводили по 0,5 мл 10% раствора глюконата кальция и 50 мг лимоннокислого натрия. Второй группе доза глюконата кальция увеличили до 1 мл 10% раствора, лимоннокислого натрия — по 50 мг на крысу. Все животные содержались в обменных клетках, где с помощью распределительной воронки собиралась моча и кал каждой крысы раздельно.

Из собранного материала готовились препараты, измерялось суточное количество мочи: 0,1 мл мочи наносили на мишень и высушивали в сушильном шкафу; суточное количество кала подсушивали в сушильном шкафу, взвешивали, затем растирали в ступке и на мишень наносили навеску в 50 мг. Для того, чтобы кал равномерно распределялся на мишени, его обильно смачивали эфиром, с жидкостью он растекался по мишени. Подсчет активности производился на установке «В» с торцовым счетчиком. Каждый препарат считали четыре раза по две минуты, а затем выводили среднеарифметические показатели. Активность каждого препарата пересчитывалась из суточное количество исследуемого материала, затем вычислялись проценты суточного выведения цезия-134 с мочой и калом по отношению к введенной дозе.

Щавелевая кислота, комплексируясь с металлами первой группы периодической системы, дает легко растворимые соединения. Цезий принадлежит к первой группе периодической системы и является наиболее активным металлом. Следовательно, он также будет реагировать со щавелевой кислотой и образовывать растворимые комплексы, легко

выводящиеся из организма. Однако при введении щавелевой кислоты крысам в брюшную полость мы не наблюдали ускорения его выведения.

У подопытных животных в местах введения щавелевой кислоты образовывались некрозы. Общее состояние крыс было тяжелым. Они не принимали пищи, шерстный покров был грязным, взъерошенным. Через сутки после введения в организм цезия-134 у подопытных животных с экскрементами выводились значительно меньшие количества цезия, чем у контрольных. На протяжении всех последующих дней наблюдения количество выводимого цезия в моче и кале подопытных крыс было меньше, чем в контроле.

За пять суток у подопытных животных выводилось в среднем с мочой и калом 29,2% (с колебаниями от 25% до 30%), а у контрольных животных — 31,3% радиоактивного цезия (с колебаниями от 30 до 33%).

Появление некрозов при внутрибрюшинном введении щавелевой кислоты побудило нас изменить метод введения ее в организм. В следующих экспериментах мы стали подмешивать щавелевую кислоту к сладкой воде, которую крысы выпивали в течение суток. Никаких побочных явлений в этом случае не наблюдалось. Поведение подопытных животных ничем не отличалось от контрольных. Выведение цезия-134 у подопытных животных в течение пяти дней ускорилось на 23%. Так, у контрольной группы выводилось в среднем 29,8% цезия-134, а у подопытных животных — в среднем 52,9%, то есть половина введенного в организм цезия-134 выводилась в течение пяти дней, в то время как период полувыведения цезия-134 у крыс равен в среднем 28 дням.

Имеющиеся многочисленные данные литературы, посвященные влиянию солей лимонной кислоты, в частности лимоннокислого натрия, на выведение радиоактивных металлов из организма, не касаются выведения цезия-134 (Хаккетт, Шуберт, Кетти). Вместе с тем, цезий, как более активный металл и как аналог натрия и калия в организме, может вытеснять натрий из соединений, занимая его место. Комплексное вещество — лимоннокислый цезий — легко растворимо и поэтому может легко выводиться из организма.

При введении лимоннокислого натрия в брюшную полость какие-либо изменения в месте инъекции не отмечены. Поведение подопытных животных не отличалось от контрольных. В первые же сутки была заметна значительная разница в выведении цезия-134 у подопытных и контрольных животных. Если у контрольных крыс вывелось 7% цезия-134, то у подопытных — 12% радиоактивного цезия. В дальнейшем за сутки у подопытных животных выводилось в среднем на 3% радиоактивного цезия больше, чем у контрольных.

В течение пяти суток у подопытных животных выводится с мочой и калом в среднем 48,3% радиоактивного цезия-134 (с колебаниями от 46% до 50%), у контрольных — 35,1% (с колебаниями от 31 до 35%). Следовательно, за пять дней количество выводимого цезия у подопытных крыс увеличилось в среднем на 15% по сравнению с контрольной группой.

При введении лимоннокислого натрия внутримышечно в местах инъекций появляются инфильтраты, а к концу наблюдения (пятые сутки) инфильтрированные ткани подвергаются некротическим изменениям. В этих случаях общее состояние подопытных крыс тяжелое. Они отказываются от пищи, шерстный покров грязный, взлохмаченный. Ускорение выведения не наблюдается.

В первые же сутки у контрольных животных выводится значительно больше радиоактивного цезия, чем у подопытных. За пять суток у подопытных животных вывелось в среднем 24,2%, а в контроле — 51% радиоактивного цезия-134.

Аналогичная картина наблюдается при введении в брюшную полость лимоннокислого натрия и щавелевой кислоты. В месте инъекции образуются некрозы, выведение цезия-134 замедляется. У контрольных животных в этом случае вывелось в среднем за пять суток 31,3% цезия-134, а у подопытных — только 15%.

Глюконат кальция и комбинация лимоннокислого натрия с глюконатом кальция для ускорения выведения радиоактивного цезия-134 применяется нами впервые. Основываясь на способности кальция уменьшать проницаемость и повышать тургор клеток (В. И. Скворцов, П. Н. Кравков, Н. В. Вершинин), мы попытались повлиять на выведение цезия с помощью глюконата кальция. Последний избран потому, что не вызывает таких раздражений тканей, как хлористый кальций. Уже через сутки после начала эксперимента количество цезия, выводимого с мочой и калом у подопытных животных, в два раза превысило количество цезия, выведенного в группе контрольных животных: так, в среднем с мочой и калом подопытных животных вывелось 22,3%, а у контрольных — только 12% радиоактивного цезия-134.

В течение пяти суток с мочой и калом у подопытных животных вывелось в среднем 55,3% цезия-134 — половина введенного в организм количества, в то время как у контрольных животных только 35,3%. Следовательно, за пять суток у подопытных животных вывелось радиоактивного цезия в среднем на 20% больше, чем у контрольных.

Увеличивая дозу вводимого глюконата кальция, удается увеличить и выведение цезия-134. В этом случае так же, как и в предыдущем опыте, в первые сутки у подопытных животных выводится в среднем вдвое больше радиоактивного цезия, чем в контроле. Если у контрольных животных с мочой и калом в первые сутки в среднем вывелось 14% цезия-134, то у подопытных — 28,7%. В течение пяти суток у контрольных животных вывелось 34% радиоактивного цезия, а у подопытных — 67% — на 23% больше, чем в контроле. В течение девяти суток у контрольных животных вывелось в среднем 40%, а у подопытных 80% радиоактивного цезия-134.

Проверив влияние глюконата кальция на выведение цезия-134, мы обнаружили, что глюконат кальция ускоряет выведение цезия незначительно — в среднем на 5% больше по сравнению с контролем. Он влияет главным образом на уменьшение проницаемости клеток и, следовательно, способствует уменьшению проникновения радиоактивного вещества внутрь клетки. Чем меньше радиоактивного вещества проникает в клетку, тем меньше образуется комплексных нерастворимых соединений его с белками. Лимоннокислый натрий, вводимый на этом фоне, связывает большие количества цезия-134 и способствует увеличению выведения его из организма.

Выводы

1. Щавелевая кислота, вводимая животным с пищей, ускоряет выведение цезия-134 в среднем на 23%.

2. Лимоннокислый натрий, введенный в брюшную полость, ускоряет выведение цезия-134 в среднем на 15% по сравнению с контролем.

3. Комбинация лимоннокислого натрия с глюконатом кальция ускоряет выведение цезия-134 в течение пяти дней на 23%, а в течение девяти дней — на 40% больше, чем в контроле.

4. Глюконат кальция, введенный в брюшную полость, ускоряет выведение цезия-134 в среднем на 5% по сравнению с контролем.

5. Некрозы и воспалительные изменения тканей тормозят выведение цезия-134 из организма.

fect is exhibited by bixin in the early phases of the
ion of linoleate. Also, bixin acts synergistically with
nce its antioxidant activity and produces a markedly
period. This activity appears to be caused by the
. A latent acceleration of the reaction without
be the result of the action of decomposition products

f the tocopherol-linoleate system, carotene produces
xygen uptake during the induction period.

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THE EFFECT OF ZIRCONIUM AND SODIUM CITRATE ON THE DISTRIBUTION AND EXCRETION OF SIMULTANEOUSLY INJECTED THORIUM AND RADIOSTRONTIUM

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The potential health hazards of radioelements taken into the body are well known (1, 2). The present study is part of a program designed to test the effect of zirconium and other salts on the metabolism of long lived radioelements. It has been shown that the early administration of zirconium salts to experimental animals previously injected with plutonium (Pu^{239}) and radioyttrium (Y^{91}) resulted in a marked increase in the urinary excretion of these radioelements and a considerable reduction of their concentration in bone (3, 4).

Thorium (Th^{230}) and radiostrontium ($Sr^{89, 90}$) are of particular interest because of the marked differences in the nature of their deposition in bone (2). Strontium is closely related metabolically to calcium and the other alkaline earths (2) and is deposited throughout the mineral structure of bone, while many elements, including thorium, plutonium, zirconium, and the rare earths, are deposited primarily in the non-mineralized areas and possibly on the superficial surfaces of the mineralized structure (1, 2). Conditions that affect the metabolism of Ca and Sr do not appear to disturb that of Pu or Y (1, 2). It appeared unlikely, therefore, that zirconium administration would affect the metabolism of Sr.

Until recently there has been little information available on the quantitative distribution and excretion of soluble thorium salts following parenteral administration (2, 6). It has been stated (2) that tracer studies and bone radioautographs showed "no significant differences between the metabolic characteristics of thorium and plutonium." Therefore, it seemed reasonable to expect that the metabolism of injected thorium followed by zirconium administration should be markedly altered, as is the case with plutonium. It is of fundamental interest, therefore, to learn that zirconium has no significant influence on Th metabolism.

Inasmuch as the citrate salt of zirconium was used in our experiments, it was necessary to study the effects of sodium citrate as a control for the action of the citrate ion. Sodium citrate administration is of value in the treatment of experimental uranium poisoning (5) and also of lead poisoning (7), but it exerts little influence on the metabolism of plutonium and yttrium (4).

EXPERIMENTAL

General Procedures—All injections were made by the intraperitoneal route. Twenty female Sprague-Dawley rats, having weights which fell between 197 and 215 gm., were injected with 1.4 ml. of a solution containing the radioisotopes of Th^{230} and $Sr^{89, 90}$. The twenty rats were divided into five groups. To the first group of four rats were administered 4.0 ml. of a zirconium citrate solution containing 12.5 mg. of Zr per ml. ½ hour after injection of the radioelements, while another group of four rats received the same amount of zirconium citrate 3 days later. These groups are referred to as "early zirconium-treated" and "late zirconium-treated," respectively. In similar fashion a third group of four rats received 4.0 ml. of a 5 per cent solution of sodium citrate ½ hour after the injection of the radioelements, and another group of two rats received the same amount of sodium citrate 3 days later. These groups are referred to as "early citrate-treated" and "late citrate-treated," respectively. The last group of six rats, the "untreated controls," received only the radioelement solution. The rats were kept two to a cage. The pooled urine and feces from each cage were collected separately, usually at daily intervals. 8 days after the injection of Th^{230} + $Sr^{89, 90}$ the animals were sacrificed with nembutal, and the liver, pancreas, femur, kidneys, spleen, mesenteric lymph node, and the lungs were removed. The rest of the animal constituted the "carcass" portion.

Radioelement Solution—A small volume of a hydrochloric acid solution of the radioelements was added to a solution of citric acid, the pH was adjusted with NaOH, and the solution diluted with distilled water. After dilution the solution used for injection had the following composition: pH 4, citric acid 1 per cent, Na^+ 0.15 M, $Sr^{89, 90}$ ~15 μc. per ml., and Th^{230} ~0.64 μc. per ml. Each animal received 1.4 ml. of this solution or a total of 1.4×10^6 counts per minute of $Sr^{89, 90}$ and 0.9×10^6 counts per minute of Th^{230}. Inasmuch as the specific activity of Th^{230} is about 43,000 disintegrations per minute per microgram or 21,500 counts per minute per microgram at 50 per cent geometry, each rat received a minimum of $0.9 \times 10^6/21.5 \times 10^3 = 42\ \gamma$ of thorium. Chemical analysis revealed the presence of about 70 γ of Th. This concentration is far below the LD_{50} value (8). A spectrographic analysis of the injection solution showed that, per ml., it contained less than 2 γ of chemical strontium or of cations other than sodium and thorium.

Zirconium and Sodium Citrate—The solution of zirconium citrate, $Na^+[ZrO(C_6H_5O_7)]^-$, was prepared as described previously (4). Each animal receiving zirconium was given 4.0 ml. of a 12.5 mg. per ml. solution or a total of 50 mg. as Zr. The sodium citrate-treated rats each received

4.0 ml. of a 5 per cent solution of sodium citr
amounts of Zr or sodium citrate administere
limits (8, 9).

Methods of Analysis—The excreta and tis
use of concentrated HNO_3 only. This was a
sample with HNO_3 and evaporating to near d
acid was then added and evaporated as befo
three to eight times, depending on the size of
matter remained, as evidenced by the light
ash. Finally, the residue was transferred wit
and diluted to volume (usually to 100 ml. fo
carcasses). The carcasses were dissolved by
centrated HNO_3, allowed to stand for at lea
as described above.

The amount of each radioelement injected
high so that a quantitative measure of its co
be made by direct deposition and subsequen
Inasmuch as Th^{230} is primarily an α emitte
(10), it was not necessary to effect any separ
aliquot (usually 0.25 ml.) of an ashed solutio
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(usually 5 ml.) for $Sr^{89, 90}$ assay were deposite
evaporated to dryness with an infra-red lam
mica window Geiger tube through a 110 mg.
The absorber was used to eliminate activi
panying Th^{230} which would otherwise be
not assayed for $Sr^{89, 90}$ until the Sr^{90} isotope
its 65 hour Y^{90} daughter.

The activity in a given sample was alwa
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into a volumetric flask, which were subse
under identical conditions. In order to ca
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from a non-radioactive animal to a sample

Results

*Comparison of Distribution and Excretio
treated Rats*—The simultaneous administra
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EXPERIMENTAL

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Citrate—The solution of zirconium citrate,
repared as described previously (4). Each ani-
s given 4.0 ml. of a 12.5 mg. per ml. solution
The sodium citrate-treated rats each received

4.0 ml. of a 5 per cent solution of sodium citrate, $Na_3C_6H_5O_7 \cdot 2H_2O$. The amounts of Zr or sodium citrate administered were well below the toxic limits (8, 9).

Methods of Analysis—The excreta and tissues were wet ashed by the use of concentrated HNO_3 only. This was accomplished by covering the sample with HNO_3 and evaporating to near dryness on a hot-plate. Fresh acid was then added and evaporated as before. This was repeated from three to eight times, depending on the size of the sample, until no organic matter remained, as evidenced by the light yellow to white color of the ash. Finally, the residue was transferred with HNO_3 to a volumetric flask and diluted to volume (usually to 100 ml. for tissues and to 2000 ml. for carcasses). The carcasses were dissolved by first being covered with concentrated HNO_3, allowed to stand for at least 12 hours, and then treated as described above.

The amount of each radioelement injected into each rat was sufficiently high so that a quantitative measure of its concentration in a sample could be made by direct deposition and subsequent counting of the radiations. Inasmuch as Th^{230} is primarily an α emitter (10) and $Sr^{89, 90}$ a β emitter (10), it was not necessary to effect any separation. For counting Th^{230} an aliquot (usually 0.25 ml.) of an ashed solution was deposited on a platinum disk, slowly evaporated to dryness by means of an infra-red lamp, and flamed. The activity was determined by counting at about 50 per cent geometry in a proportional α-counter unresponsive to β-particles. Samples (usually 5 ml.) for $Sr^{89, 90}$ assay were deposited in a 10 ml. porcelain capsule, evaporated to dryness with an infra-red lamp, and counted by means of a mica window Geiger tube through a 110 mg. per sq. cm. aluminum absorber. The absorber was used to eliminate activity contributed by the accompanying Th^{230} which would otherwise be recorded. The samples were not assayed for $Sr^{89, 90}$ until the Sr^{90} isotope had regained equilibrium with its 65 hour Y^{90} daughter.

The activity in a given sample was always referred to that of samples from the original injection solutions obtained from "dummy" injections into a volumetric flask, which were subsequently mounted and counted under identical conditions. In order to cancel effects due to absorption and other factors, a separate standard for each tissue was prepared by addition of an equivalent amount of an ashed solution of the same tissue from a non-radioactive animal to a sample from the "dummy" injection.

Results

Comparison of Distribution and Excretion of Th^{230} and $Sr^{89, 90}$ in Untreated Rats—The simultaneous administration of the two radioelements permits a direct comparison of their behavior in the same animal. The

data tabulated in Table I illustrate the striking differences in the excretion and distribution of Th and Sr after 8 days. Sufficient time having elapsed for the uptake in bone to have reached a maximum (2, 11), it was found that the concentration of injected Th was considerable in all the soft tissues, reaching 30 per cent of the injected dose in liver alone, while that of Sr was negligible, *i.e.* less than 0.02 per cent. On the other hand, the concentration of Sr in the bone was 3 times higher than that of Th.

The total urinary and fecal excretion of Sr was about twice that of Th. Urinary excretion of both radioelements was highest during the first 24 hours after injection. It then dropped sharply during the subsequent days, especially in the case of thorium. By the 8th day the urinary excre-

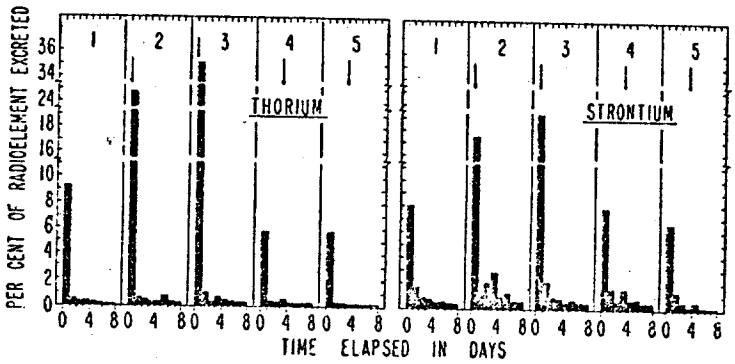

FIG. 1. The effect of zirconium and sodium citrate administration to rats on the urinary excretion of injected Th^{230} and $Sr^{89,90}$, respectively. Each arrow represents a single intraperitoneal injection of zirconium or sodium citrate. The numeral within each panel represents the following treatments: Panel 1 controls, Panel 2 early zirconium citrate, Panel 3 early sodium citrate, Panel 4 late zirconium citrate, Panel 5 late sodium citrate.

tion of strontium had reached about 0.4 to 0.5 per cent of the injected dose per day, while that of thorium had diminished to about 0.05 per cent. The day by day urinary excretion of these elements is shown in Fig. 1. The daily fecal excretion of Sr was quite erratic, while that of Th proceeded at a fairly constant rate of about 0.5 to 1.5 per cent a day.

Effect of Treatment—The early administration of either zirconium or sodium citrate caused the urinary excretion of Th to increase from about 9 to about 30 per cent of the injected dose (Fig. 1) and that of Sr from 5 to about 18 per cent during the subsequent 24 hour period (Fig. 1). Some sustained increase in Sr excretion following early Zr administration, however, seems to have occurred. Injections of sodium citrate 72 hours after exposure to Th and Sr had no effect on their urinary output, while zirconium citrate appeared to have a slight effect on Sr excretion and none on Th (Fig. 1). It must be kept in mind, however, that a 3-fold increase, for

example, in the urinary excretion o
usually constitutes an appreciable
relatively unimportant when the no
low levels.

The excretion of Sr or Th in the f
administration of zirconium or sodi

Since the major part of the incr
followed either early sodium or zirco
the liver, it must be concluded that
observed effects (Fig. 2 and Table I)
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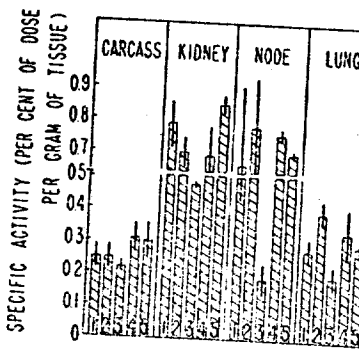

FIG. 2. The effect of zirconium and sodi
tration of injected Th^{230} in the tissues of rat
receiving the Th^{230}. The numerals inside t
ments: Panel 1 controls, Panel 2 early zirconi
Panel 4 late zirconium citrate, Panel 5 late
resent the average deviation among the mem

mesenteric lymph node, and pancreas
sodium citrate. The increased strontiu
the skeleton primarily. It appears lik
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toto contain about 35 per cent of tracer
injection (12).

The results described above are in n
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to 50 per cent of the injected dose durin
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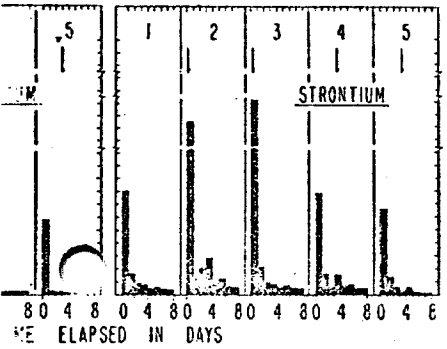

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mind, however, that a 3-fold increase, for

example, in the urinary excretion of a radioelement shortly after exposure usually constitutes an appreciable fraction of the body content but is relatively unimportant when the normal excretion rate has dropped to very low levels.

The excretion of Sr or Th in the feces was unaffected at any time by the administration of zirconium or sodium citrate.

Since the major part of the increased excretion of the thorium which followed either early sodium or zirconium citrate administration came from the liver, it must be concluded that the citrate ion is responsible for the observed effects (Fig. 2 and Table I). Thorium excretion was also accomplished at the expense of the other soft tissues, namely the kidneys, spleen,

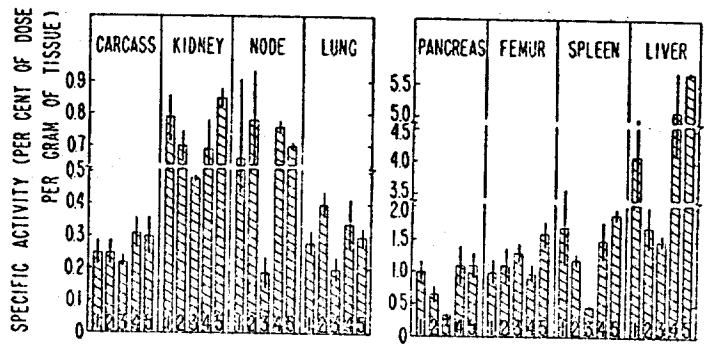

FIG. 2. The effect of zirconium and sodium citrate administration on the concentration of injected Th^{230} in the tissues of rats. The rats were sacrificed 8 days after receiving the Th^{230}. The numerals inside the panels represent the following treatments: Panel 1 controls, Panel 2 early zirconium citrate, Panel 3 early sodium citrate, Panel 4 late zirconium citrate, Panel 5 late sodium citrate. The vertical lines represent the average deviation among the members of each group of rats.

mesenteric lymph node, and pancreas, following the administration of sodium citrate. The increased strontium excretion seemed to come from the skeleton primarily. It appears likely that the muscle and skin contributed some of the increased Sr excretion inasmuch as these tissues *in vivo* contain about 35 per cent of tracer Sr 30 minutes after intraperitoneal injection (12).

The results described above are in marked contrast to those obtained with Pu under the same conditions (4). Thus, early zirconium citrate administration increased the urinary excretion of Pu from the normal 1 to 50 per cent of the injected dose during the subsequent 24 hours, while sodium citrate caused a 2-fold increase only. In addition the bone content of Pu was found to be reduced about 6-fold as a result of zirconium treatment, while the soft tissue concentrations were essentially unchanged. Furthermore, the administration of zirconium citrate 3 days after the in-

jection of Pu raised the urinary excretion level from 0.1 to 5 per cent of the injected dose.

TABLE I

*Effect of Zirconium and Sodium on Distribution and Excretion of Simultaneously Injected Thorium and Radiostrontium in Rats**

Tissues	Per cent injected dose per organ									
	Untreated controls		Early zirconium, citrate-treated		Early sodium, citrate-treated		Late zirconium, citrate-treated		Late sodium, citrate-treated	
	Th	Sr	Th	Sr	Th	Sr	Th	Sr	Th	Sr
Carcass	47.4 ±6.9	67.0 ±5.4	48.2 ±6.2	53.0 ±4.4	41.0 ±2.8	56.9 ±9.9	51.1 ±9.2	66.0 ±4	49 ±6	70.5 ±3.3
Skeleton†	17.8 ±3.6	52.8 ±6	20 ±2	40.8 ±4.1	22 ±4	44.6 ±10	17.2 ±1.8	46.6 ±2	28 ±1	50 ±0.2
Liver	29.6 ±6.8	0.015 ±0.002	14.4 ±2.3	0.020 ±0.006	9.8 ±2.0	0.010 ±0.001	31.8 ±4.5	0.018 ±0.002	33.8 ±1.8	0.02[illegible] ±0.00[illegible]
Kidneys	1.2 ±0.08	<0.02	1.1 ±0.12	<0.02	0.74 ±0.12	<0.02	1.0 ±0.17	<0.02	1.2 ±0.1	<0.02
Spleen	1.0 ±0.2	<0.01	0.66 ±0.02	<0.02	0.27 ±0.01	<0.01	0.85 ±0.12	<0.02	0.82 ±0.06	<0.02
Mesenteric lymph node	0.30 ±0.06	<0.01	0.41 ±0.10	<0.01	0.12 ±0.08	<0.01	0.29 ±0.02	<0.01	0.26 ±0.20	<0.01
Pancreas	1.0 ±0.2	<0.01	0.61 ±0.10	<0.01	0.33 ±0.08	<0.01	1.1 ±0.3	<0.01	0.93 ±0.20	<0.01
Lung	0.37 ±0.04	<0.02	0.53 ±0.04	<0.02	0.29 ±0.04	<0.02	0.45 ±0.10	<0.02	0.43 ±0.09	<0.02
Urine	10.6 ±4	13.5 ±4	27.4 ±6	27.1 ±8	36.8 ±3	25.1 ±5	6.3 ±0.5	14.2 ±0.5	6 ±3	9.70 ±4
Feces	7.7 ±2	16.7 ±1.5	5.5 ±2	19.0 ±2.1	9.6 ±3	16.2 ±2.2	6.2 ±2	17.5 ±0.6	6 ±3	17.4 ±3

* Values equated to 100 per cent (actual recoveries for Th = 94.5 ± 8.2 per cent and for Sr = 90 ± 4 per cent). The rats were sacrificed 8 days after the intraperitoneal administration of Io^{230} (Th) and $Sr^{89,90}$. Each value is an average of results obtained from six rats for the untreated controls, four rats each for the early sodium citrate, early zirconium citrate, and late zirconium citrate-treated animals, and two rats for the late sodium citrate group. Further details are given in the experimental sections of the text.

† The concentrations of radioelement in the skeleton were calculated by multiplying the femur content by the somewhat arbitrary factor of 20.

DISCUSSION

Action of Sodium Citrate—The action of early sodium citrate administration in minimizing the deposition of thorium in the soft tissues with resulting increase in the urinary excretion is related to the complexing action of the citrate ion accompanied by a concomitant increase in the

diffusibility of the Th. Most cations in t
fusible and non-diffusible forms. The no
a combination of the cation with protein
particles with colloidal properties as a co
tion with anions to form salts of low sol
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have been proposed to explain the action of
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Once an element such as thorium, whic
organic solution above a pH of 3.5, has be
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of an element such as Th once it has formed
the relative ineffectiveness of sodium citrat
of Th when administered several days later.

Of further interest is the fact that the co
the spleen, mesenteric lymph node, and pa
by early zirconium citrate administration;
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lated to the fact that in zirconium citrate th
is not immediately available because it is bo
the time necessary for the zirconium citrat
intraperitoneal injection, the mesenteric lyn
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free the bound citrate, whereupon it is avai
effect through complexing action on the Th
et in the liver partially by conversion to gl

The uptake of strontium and the other al
phosphate ion is most easily interpreted as bei
18–20) with the bone acting as either an anio
taining a variety of functional groups. We c
change with strontium by the relation

$$CaA + Sr^{++} \rightleftharpoons SrA + Ca$$

[1] Unpublished experiments.

urinary excretion level from 0.1 to 5 per cent of

TABLE I

*Sodium on Distribution and Excretion of Simultaneously horium and Radiostrontium in Rats**

Per cent injected dose per organ

rly zirconium, citrate-treated		Early sodium, citrate-treated		Late zirconium, citrate-treated		Late sodium, citrate-treated	
Th	Sr	Th	Sr	Th	Sr	Th	Sr
S.2	53.0	41.0	56.9	51.1	66.0	49	70.5
6.2	±4.4	±2.8	±9.9	±9.2	±4	±6	±3.3
0	40.8	22	44.6	17.2	46.6	28	50
2	±4.1	±4	±10	±1.8	±2	±1	±0.2
4.4	0.020	9.8	0.010	31.8	0.018	33.8	0.02
2.3	±0.006	±2.0	±0.001	±4.5	±0.002	±1.8	±0.00
1.1	<0.02	0.74	<0.02	1.0	<0.02	1.2	<0.02
0.12		±0.12		±0.17		±0.1	
0.66	<0.02	0.27	<0.01	0.85	<0.02	0.82	<0.02
0.02		±0.01		±0.12		±0.06	
0.41	01	0.12	<0.01	0.29	<0.01	0.26	<0.01
0.10		±0.08		±0.02		±0.20	
0.61	<0.01	0.33	<0.01	1.1	<0.01	0.93	<0.01
0.10		±0.08		±0.3		±0.20	
0.53	<0.02	0.29	<0.02	0.45	<0.02	0.43	<0.02
0.04		±0.04		±0.10		±0.09	
4	27.1	36.8	25.1	6.3	14.2	6	9.70
	±8	±3	±5	±0.5	±0.5	±3	±4
5	19.0	9.6	16.2	6.2	17.5	6	17.4
	±2.1	±3	±2.2	±2	±0.6	±3	±3

er cent (actual recoveries for Th = 94.5 ± 8.2 per cent t). The rats were sacrificed 8 days after the intraper ^{230} (Th) and $Sr^{89, 90}$. Each value is an average of r for the untreated controls, four rats each for the early nium citrate, and late zirconium citrate-treated an late sodium citrate group. Further details are give of the text.

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DISCUSSION

ple—The action of early sodium citrate admini deposition of thorium in the soft tissues with urinary excretion is related to the complexin accompanied by a concomitant increase in the

diffusibility of the Th. Most cations in the body exist as a mixture of diffusible and non-diffusible forms. The non-diffusible fractions result from a combination of the cation with proteins, or because of the formation of particles with colloidal properties as a consequence of hydrolysis or reaction with anions to form salts of low solubility. An increase in the diffusible fraction at the expense of the non-diffusible parts is reflected in a lower deposition in the soft tissues comprising the reticuloendothelial system and an increase in the urinary excretion because the rate of filtration through the glomerulus of the kidney is enhanced. These considerations are supported by the fact that citrates form soluble complex ions with thorium (13) and by ultrafiltration studies in which the passage of Th from blood serum through Visking membranes was markedly increased following the addition of sodium citrate.[1] Similar concepts have been proposed to explain the action of sodium citrate and other complexing axions in the metabolism of uranium (14, 15) and lead (16).

Once an element such as thorium, which precipitates from simple inorganic solution above a pH of 3.5, has been deposited in the body, it is very difficult to increase its subsequent rate of elimination. This is probably related to the well known chemical fact that very large (unphysiological) amounts of the citrate anion are needed to dissolve the hydroxide of an element such as Th once it has formed. This phenomenon explains the relative ineffectiveness of sodium citrate in promoting the excretion of Th when administered several days later.

Of further interest is the fact that the concentration of injected Th in the spleen, mesenteric lymph node, and pancreas was clearly unaffected by early zirconium citrate administration; yet early sodium citrate administration caused a marked reduction. Such a result is probably related to the fact that in zirconium citrate the citrate part of the molecule is not immediately available because it is bound to zirconium. Thus in the time necessary for the zirconium citrate to be absorbed following intraperitoneal injection, the mesenteric lymph node, spleen, and pancreas are not in contact with free citrate. Subsequent metabolic processes free the bound citrate, whereupon it is available to exert a solubilizing effect through complexing action on the Th. Free citrate, however, is lost in the liver partially by conversion to glycogen (17).

The uptake of strontium and the other alkaline earths as well as the phosphate ion is most easily interpreted as being an ion exchange reaction (18–20) with the bone acting as either an anion or cation exchanger containing a variety of functional groups. We can represent the cation exchange with strontium by the relation

$$CaA + Sr^{++} \rightleftharpoons SrA + Ca^{++} \qquad (1)$$

[1] Unpublished experiments.

in which, for simplification, we assume that A is a composite anion of all the functional groups in bone with a valence of -2. The capacity of bone for cation exchange can be expressed in terms of the total milliequivalents of replaceable calcium. The distribution coefficient, K_d, for a given cation adsorbed by bone is given by the expression (21)

$$K_d = \frac{M_s}{M_1} \times \frac{v}{m} \tag{2}$$

in which M_s and M_1 are the fractions of the adsorbed cation in the bone and liquid phases respectively, and v is the volume of solution in contact with m mg. of bone. The ratio $M_s:M_1$ is expressed conveniently as (per cent adsorbed)/(100 per cent minus per cent adsorbed). By employing the same reasoning used with resinous cation exchangers (21), it can be shown that, to a first approximation, K_d is directly proportional to the mole fraction of exchangeable Ca^{++} in bone. Therefore K_d, and hence the amount of $Sr^{89,90}$ exchanged, will remain constant as long as the mole fraction of Ca and other exchangeable cations in bone remain unchanged. Since a gm. of bone contains about 1 mM of exchangeable calcium, tracer levels of $Sr^{89,90}$, *i.e.* $\sim 10^{-10}$ M, cannot affect K_d, regardless of the extent to which the tracer Sr is adsorbed. In the *in vitro* experiments of Falkenheim *et al.* (19), 50 mg. of bone ash were equilibrated with 25 ml. volumes of solution-containing Ca^{++}. Their bone sample presumably contained about 0.05 mM of exchangeable calcium. When the contacting solution contained less than 10^{-4} M, *i.e.* <0.0025 mM of tracer alkaline earth, no appreciable change in K_d could be expected. However, a solution 10^{-3} M, equivalent to 0.025 mM, if adsorbed to the same extent as tracer levels, $\sim$75 per cent or more as found for Sr and Ca (19, 22), would markedly reduce the mM of Ca in bone, and hence a sharp drop in the per cent adsorption must take place at about 10^{-3} M, in agreement with the experiments on bone (18, 19).

The preceding calculations explain why the injection of 0.01 to 10 μc. of carrier-free $Sr^{89,90}$ in the experiments of Murray and Bloom (23) resulted in no change in the per cent uptake.[2] In some experiments of Copp and Greenberg (24) it was thought that the injection of non-radioactive "carrier" strontium might dilute and wash out radioactive strontium. In view of the large amount of exchangeable calcium in bone it can be calculated from equation (2) that it would require about 60 mg. of Sr in a 200 gm. rat to affect the uptake of Sr appreciably. This was, indeed, found to be the case, but inasmuch as this amount of Sr is lethal or near lethal it is of no practical value.

[2] We are, of course, disregarding any effects due to the radiation itself.

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SUMMA

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(1947).
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ng a ffects due to the radiation itself.

Elements such as Th, Pu, and Zr, because of their chemical properties, are most probably retained by bone through an irreversible type of non-specific surface adsorption. Therefore, the uptake and elimination of these elements by bone, in analogy to similar inorganic systems (21), should be unaffected by changes in bone resulting from age, diet, or other ordinary metabolic changes. A fuller discussion of these points together with pertinent experimental data will be given in a subsequent paper.

SUMMARY

1. The distribution and daily excretion of tracer levels of injected thorium and strontium were studied in rats receiving zirconium or sodium citrate intraperitoneally ½ hour after the administration of the radio-elements ("early" treated) or 3 days later ("late" treated). One group of rats received no other treatment.

2. In contrast to previous studies on plutonium and yttrium, zirconium citrate had no specific effect on Th or Sr metabolism other than that associated with the citrate part of the molecule.

3. The early administration of zirconium or sodium citrate resulted in about a 3-fold increase in the urinary excretion of Th and a 2-fold increase in the urinary excretion of Sr during the following 24 hours. No effect on the fecal excretion was found. The Th concentration in the liver, mesenteric lymph node, pancreas, and spleen was markedly reduced as a result of early sodium citrate administration. No significant changes in the metabolism of Th or Sr were found in the late treated groups.

4. The deposition of Th per gm. was highest in the soft tissues, particularly in the liver, while Sr accumulated mainly in the skeleton.

5. An interpretation of the results in terms of ion exchange, surface adsorption, and complex ion formation was presented.

We wish to express our appreciation to Rosie Hunter for the mounting and counting of many of the numerous samples required by this work.

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GROUPS INVOLVED IN THE ZIM
REACTION

By H. W. MARI

(From the Department of Biochemistry, Sout
University of Texas,

(Received for publication, J

Colorimetric methods for the quantitativ
pounds have been developed, based on the
cochemical properties. Such tests include
for androgenic assay and the Kober (2)
Modifications of each of these have been
Cohen and Bates (4), Szego and Samuels (5)
Many of these modifications are for the det
androgens obtained from the extract of uri
The value of these variations lies in the fact
in the perhydro-1, 2-cyclopentanophenanthre
are involved. The color development in the
mermann (1) and others) depends upon a ca
methylene carbon (—CH_2—CO—). For this
related to 17-ketosteroids will give positive resu
that the group —CH_2—CO—CH_2— found in
rily the one found in perhydro-1, 2-cyclopentan
giving a positive Zimmermann. If this were t
give a positive reaction. These data do not co

Marrian (7) has postulated that 16-ketoestro
color developed in the Kober reaction, and that
a positive Kober test. This was before 16-ke
and tested. Marlow (8) showed that little or n
16-ketoestrone is treated with the Kober or Zim
pared to other estrogens.

EXPERIMENTAL

The compounds used in these experiments a
pounds of proved structure. The quantity treat
reagent has an arbitrary concentration of 72 γ,
the Kober reagents has 30 γ. Some of these co
ketoestrone, etc.) have not been reported as subj
Zimmermann tests, since they have only been syn
tures established very recently. These compound

Source: Arch. Institut Pasteur d'Algerie, XXX, No.1 March 1952, p.55-6

The Toxicity of Sodium Citrate for Small Laboratory Rodents

By Edmond Sergent and Mme. A. Poncet

In laboratory experiments it is sometimes useful to make the blood to be inoculated in animals incoagulable. For this reason we have checked the toxicity of sodium citrate for the small laboratory rodents - guinea pigs, white mice, white rats.

The salts used in our experiments were: Purified crystallized neutral sodium (trisodium) citrate of Rhone-Poulene, and Sodii citras, Sodium citrate, U.S.P., of Pfizer New York. The results were the same with either sample of citrate.

The sodium citrate was used in 5% solutions. IT was verified that a 3.5% solution produced the same effect.

The results of the experiments were the same for the three kinds of rodents.

The isotonic solution of 5% sodium citrate was injected under the skin or into the peritonium. It was also administered by ingestion.

Per kg of animal	Injection subcutaneous	Intraperitoneal	Ingestion
0.25 g	Non-toxic	Non-toxic	Non-toxic
0.50 g	Highly toxic	Toxic	"
1.24 g	Extremely toxic Frequent death	Very toxic Case of death	"

The toxic effects manifest themselves immediately after the injection. Death, when it occurs, comes within 10 minutes. The survivors, after having shown nervous disorders: unrest, shivers, depression- stupor, closed eyes, dyspnea - tonic and clonic convulsions, recover completely in several hours.

In all experiments, the subcutaneous injection had a much more toxic

effect than intraperitoneal injection at the same doses. Doses which are lethal when introduced parenterally are not toxic at all when given by mouth.

A physiological solution, injected as control, under the skin or into the peritonium, at the same doses as the solutions of citrated water, caused no disorder of any kind.

The toxic dose of sodium citrate for man is, according to Bagdassarow, cited by G. Jeanneney and G. Ringenbach (1), 15 to 20 grams for a man weighing 70 kilograms, or 0.21 to 0.28 g per kilogram.

Sodium citrate seems, therefore, to have the same toxicity for the small laboratory rodents as for man. It is wise, in laboratory experiments, not to exceed the dose of 0.25 g per kilogram of animal.

Institut Pasteur d'Algerie

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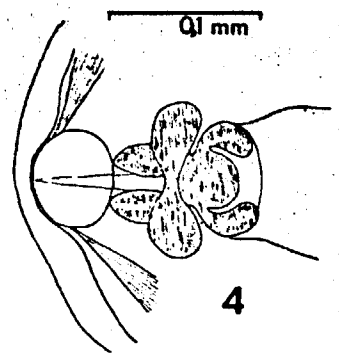
(Translated by Carl Demrick Associates,Inc/LH/t)



Tenuiproboscis setyenti nov. sp.
4, sphincter vaginal et bulbe ; — 5, œuf.

Hôte : *Canis familiaris.*
Habitat : intestin grêle.
Localité : Alger. Algérie.
Date : août 1951.

Spécimens déposés dans la collection de l'Institute of Parasitology, Macdonald College of McGill University.

Institut de parasitologie du Collège Macdonald,
l'Université MacGill, Montréal,
et Institut Pasteur d'Algérie.

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DE LA TOXICITÉ DU CITRATE DE SOUDE POUR LES PETITS RONGEURS DE LABORATOIRE

par Edmond SERGENT et Mme A. PONCET

Dans les expériences de laboratoire, il est parfois utile de rendre incoagulable le sang que l'on veut inoculer à des animaux. C'est pourquoi nous avons vérifié la toxicité du citrate de soude pour les petits rongeurs de laboratoire : cobayes, souris blanches, rats blancs.

Les sels utilisés dans nos expériences ont été : le citrate de sodium neutre cristallisé (trisodique) purifié, de la marque Rhône-Poulenc, — Sodii citras, Sodium citrate, U.S.P., marque Pfizer New-York. Les résultats constatés ont été les mêmes avec l'un et l'autre échantillon de citrate.

Le citrate de soude était employé en solutions à 5 %. On a vérifié qu'une solution à 3,5 % produisait le même effet.

Les résultats des expériences ont été les mêmes pour les trois espèces de rongeurs.

La solution isotonique de citrate de soude à 5 % a été injectée sous la peau ou dans le péritoine. On l'a également administrée par ingestion.

Par kilogramme d'animal	Injection		Ingestion
	sous-cutanée	intrapéritonéale	
0 g 25	Non toxique	Non toxique	Non toxique
0 g 50	Fort toxique	Toxique	
1 g 24	Extrêmement toxique Mort fréquente	Très toxique Des cas de mort	

Les effets toxiques se manifestent immédiatement après l'injection. La mort, quand elle se produit, survient dans les 10 minutes.

Reçu pour publication le 5 janvier 1952

Les survivants, après avoir présenté des troubles nerveux : inquiétude, frissons, abattement, — stupeur, yeux clos, dyspnée, — convulsions toniques et cloniques, guérissent complètement en quelques heures.

Dans toutes les expériences, l'injection sous-cutanée a eu un effet bien plus toxique que l'injection intrapéritonéale aux mêmes doses. Des doses, qui sont mortelles quand elles sont introduites par voie parentérale, ne sont aucunement toxiques quand elles sont données par la bouche.

Une solution d'eau physiologique, injectée à titre de témoin, sous la peau ou dans le péritoine, aux mêmes doses que les solutions d'eau citratée, n'a donné lieu à aucun trouble.

oOo

La dose toxique du citrate de soude pour l'homme est, d'après Bagdassarow, cité par G. Jeanneney et G. Ringenbach (1), de 15 à 20 grammes pour un homme du poids de 70 kilogrammes, soit 0 g 21 à 0 g 28 par kilogramme.

Le citrate de soude paraît donc avoir la même toxicité pour les petits rongeurs de laboratoire que pour l'homme. Il est prudent, dans les expériences de laboratoire, de ne pas dépasser la dose de 0 g 25 par kilogramme d'animal.

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SUR L'ACCLIMATEMENT DES GAMBOUSES EN ALGÉRIE

par E. Collignon

Importés en Algérie en 1926, par Edm. et Et. Sergent (1), les gambouses ou gambusies (*Gambusia holbrooki*), poissons ovovivipares de petite taille, originaires du Texas (Etats-Unis d'Amérique), s'y sont très bien acclimatés. Leur appétit presque insatiable pour les larves de Culicides, en particulier pour les larves d'Anophèles, permet de les utiliser comme auxiliaires de la lutte antipaludique, à laquelle ils rendent les services les plus utiles et les moins onéreux. Leur communauté d'habitat aquatique avec les larves d'*Anopheles maculipennis* les met à même, en effet, de satisfaire leur voracité : comme celles-ci, ils recherchent les eaux ensoleillées et peu courantes, se tiennent au voisinage de la surface liquide, et, grâce à leur petite taille, peuvent circuler facilement dans les fourrés de plantes aquatiques immergées. La température de l'eau propice à leur activité et à leur multiplication coïncide sensiblement avec celle qu'exige le développement des larves d'*A. maculipennis*.

L'adaptation des gambouses aux eaux douces des plaines ou des collines littorales de l'Algérie est aujourd'hui bien établie (2). Nous avons eu l'occasion d'en observer un exemple assez remarquable en montagne, dans un petit lac de la Kabylie (département d'Alger).

Ce lac, « Agoulmim oumerbout » ou « Etang du genévrier », se trouve à 160 km. d'Alger, à trois cents mètres environ au Nord de la route nationale n° 12 qui se détache, après Ménerville, de la route nationale d'Alger à Constantine pour aller vers Bougie, et à hauteur de la borne hectométrique 103,6, c'est-à-dire à 8 km. à l'Est de Yakouren et à un kilomètre à l'Ouest du col de Tagma (v. la carte). Il est situé sur la pente nord-ouest du massif d'Akfadou, à trois kilomètres de la ligne de crêtes, au Nord de l'extrémité est de la forêt des Beni R'obri, boisement peu dense, constitué par des chênes à feuilles de châtaignier (*Quercus afares*), et coupé par de larges clairières herbeuses.

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377

PARALYZING EFFECTS OF SODIUM CITRATE ON THE CARDIAC VAGUS AND ON HEART MUSCLE OF THE CAT[1]

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Received for publication July 13, 1936

After the discovery that the cardiac vagus mechanism in the turtle was temporarily paralyzed upon the injection of suitable amounts of a solution of sodium citrate (or of sodium oxalate) into the circulating blood, and that the paralysis could be prevented by the injection of a solution of calcium chloride either along with or immediately before the injection of the sodium citrate (1), it seemed pertinent to investigate any possible similar effects of these salts when introduced into the circulation of a mammal. Such a study seemed especially desirable with respect to sodium citrate, since it is so often introduced into the circulatory system of man in blood transfusions and causes there, not infrequently, an unfavorable reaction—the "citrate reaction" (2), Drinker and Brittingham (3), and others.

Accordingly, a series of experiments using sodium citrate was run on twenty-five cats as subjects. The very first experiments showed that a 10 per cent solution of sodium citrate (pure crystals) could be injected into the blood of this mammal in sufficient amounts to cause temporary paralysis of the cardiac vagus mechanism just as had been found in case of the turtle. As might be expected, however, we soon found there was greater danger, in case of the mammal, of causing death from an overdose of the sodium citrate, since a slightly larger dose than was necessary to completely paralyze the vagus tended, also, to cause a slowing

[1] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council of Pharmacy and Chemistry, American Medical Association.

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of the heart by a paralyzing effect upon the heart muscle itself, and to depress (or stop) the respiratory movements after blood pressure had been critically lowered. Very early, therefore, we settled upon a steady injection rate of the sodium citrate solution into the saphenous vein of the cat at between 1.0 and 1.5 cc. per minute.

A graphic record of blood pressure was obtained (from the right carotid artery) which showed also the pulse and the respiratory waves, except when breathing movements were slow and, at the same time, very shallow. In some experiments venous pressure was obtained, also, by means of a water manometer connected to a long cannula inserted through the external jugular vein down into the superior vena cava. Clotting was controlled in the carotid cannula by a 50 per cent solution of sodium hyposulphite, and in the venous cannula by a solution of heparin (40 mgm. to 5 cc. of Ringer's) in the pressure tubes connecting the respective cannulae with the recording manometers. Both vagi were always exposed and experiments were carried out with these nerves intact and also with both nerves severed.

Pentobarbital and ether were the anesthetics tried, but most of the experiments were carried out on decerebrate animals after recovery from ether anesthesia. When an animal was in good condition, recovery of the vagus from paralysis appeared to be about complete in twenty-five to thirty minutes, as judged by the amount of inhibition of the heart obtained upon maximal faradic stimulation of the vagus nerves. After that, second and even third trials were sometimes made with sodium citrate to paralyze the cardiac vagus mechanism and then again obtain recovery.

Obviously, when comparison of average results is to be made, the injection periods should be, as nearly as possible, equal in the different experiments and the test for paralysis of the vagus should be made as soon as possible after completion of the sodium citrate injection, to avoid differences in degree of recovery in different experiments. After our first preliminary experiments, we found a criterion by which we could reduce the number of injections of the sodium citrate solution necessary to paralyze

the vagus to one or two. Having begun our injection, as it proceeded (after the first slight rise in blood pressure), when the pressure was slowly falling and the respiration began to be depressed, we watched the graphic record for the first signs of irregularity or sudden marked slowing of the heart rate. Then the injection was stopped at once. After a few seconds at this slower rate, the heart would again, almost as suddenly, resume its former rate. Following that we usually found the vagus inhibitory mechanism to be paralyzed (or almost completely paralyzed) for a short while, before its recovery set in. Since the first trials of different series of experiments are probably the best from which to make comparison of dosage required to paralyze the vagus (or to kill), only "first trials" are used in the following list of dose ranges and averages.

In four experiments using pentobarbital as the anesthetic the range of the sodium citrate dosage necessary to paralyze the cardiac vagus was from 1.1 to 1.9 cc. of the 10 per cent sodium citrate solution per kilogram of body weight—the average being 1.5 cc.; in seven experiments using ether anesthesia the range of dosage was from 0.91 to 1.66 cc.—the average being 1.18 cc.; and in four experiments with decerebrate cats the range was from 1.58 to 2.26 cc.—the average being 2.00 cc. We had eight experiments with decerebrate cats that were bled into measured quantities of the 10 per cent sodium citrate solution after which the citrated blood was transfused into the animal from which it had been drawn. The range of sodium citrate dosage necessary to paralyze in these experiments was from 1.00 to 2.1 cc.—the average being 1.5 cc. One first trial experiment, in which the cat had been injected with 0.25 mgm. per kilogram of physostigmine, under ether anesthesia, required 2.1 cc. of the 10 per cent sodium citrate to paralyze the cardiac vagus. However, the decerebrate but normal animals (i.e., cats not bled) clearly required the largest average dose of any group. Likewise, when all the injection trials (including second and third trial experiments) were considered, it seemed equally clear that the decerebrate (but otherwise normal) cats could endure a correspondingly larger dosage of the sodium citrate solution than could either the

anesthetized or the decerebrate (but bled) cats, before direct paralysis of the heart muscle and depression of the respirations to the critical point were brought about.

Loewi ((4) and in later papers) demonstrated the production of a "vagus inhibitory substance" in the frog's heart upon stimulation of the vagus nerve; and later, Loewi and Navratil (5) identified this substance as a choline ester—probably acetylcholine. Moreover, Feldberg and Krayer (6) established a similar finding for the hearts of the dog and the cat. Alles (7) has reviewed the literature on this subject. The evidence indicates that the "vagus substance" is liberated at the *terminations* of the postganglionic vagus fibers in the heart. We have mentioned evidence that the injected amounts of sodium citrate necessary to paralyze the vagus mechanism in the turtle *did not paralyze nerve cell bodies and their axones*—viz., postganglionic neurons were still functional on direct stimulation. Moreover, reflexes to voluntary muscles could still be evoked in the turtle; and likewise, in the cat, after sufficient sodium citrate had been injected into the blood just to paralyze, completely, the vagus mechanism, we found that reflexes to voluntary muscles were easily evoked. Loewi and Navratil (8) showed that physostigmine retards the destruction of the "vagus substance" at the termination of the vagus fibers in the heart. We injected proper doses of physostigmine three minutes before introducing sodium citrate into the blood of the cat and found that the physostigmine did not prevent the citrate from finally completely paralyzing the cardiac vagus. With all these findings in mind, therefore, and accepting the view that the vagus substance is identical with (or quite similar in its action to) acetylcholine, we decided to inject sodium citrate solution into the saphenous vein of a decerebrate cat until the cardiac vagus was just completely paralyzed (no slowing of the heart being produced by maximal faradic stimulation of the vagus), and then by introducing a previously found effective dose of acetylcholine into the circulation, to thus test whether it would slow the heart as it did before sodium citrate had been used. If the introduced acetylcholine should still prove to be effective in temporarily inhibiting the heart, while stimulation of the vagus

(preganglionic fibers) was not effective, this would be good indirect evidence that the seat of citrate paralysis of the cardiac vagus in the cat was at the synapse between pre- and postganglionic neurons—and not at the connections of postganglionic fibers with the heart muscle.

Acetylcholine was used in experiments on five cats as outlined above. In one experiment, for example, after the vagus mechanism had been completely paralyzed by sodium citrate so that prolonged stimulation of the right vagus with maximal faradic shocks caused practically no slowing of the heart either before or after an intravenous injection of 0.4 cc. of 1:10,000 acetylcholine, the injected drug, nevertheless, caused the same slowing of the heart and fall in blood pressure as before the vagus had been paralyzed. After about forty-five minutes, then, we found the vagus recovered so that it inhibited the heart again on stimulation just as it had done before the citrate paralysis. This experiment, confirmed by others of the series, gives indirect evidence that the vagus inhibitory substance is not produced during stimulation of the citrate-paralyzed vagus for the reason that the seat of paralysis is at the synapses between pre- and postganglionic neurons, just as direct evidence already cited proved it to be in the citrate-paralyzed vagus of the turtle. Shafer (former citation) had some evidence that larger doses of sodium citrate than the minimum necessary to completely paralyze the synapses between pre- and postganglionic neurons of the cardiac vagus could paralyze the postganglionic mechanism, also, of the turtle, for a short time. We obtained no certain evidence on this point for the cat—the larger dose always endangered the life of the cat so that we could not test with acetylcholine at what we believed to be the right moment.

We tested, several times, to find whether synapses in a sympathetic ganglion of the autonomic system were paralyzed by sodium citrate introduced into the blood. Namely, we stimulated preganglionic sympathetic fibers in the cephalad end of the severed vago-sympathetic nerve cord of the cat and observed the degree of retraction of the nictitating membrane at the time when sufficient sodium citrate had been injected into the blood

to completely paralyze the cardiac vagus. Under that dosage of sodium citrate, we observed no case of paralysis of the sympathetic nervous mechanism controlling the smooth muscle fibers that cause full retraction of the nictitating membrane. On applying faradic stimulation to the vago-sympathetic trunk, the membrane always responded by a retraction movement. This result was to be expected, perhaps, if acetylcholine is the normal excitor substance of postganglionic neurons at synapses in sympathetic ganglia, because as our own results have already shown, injected acetylcholine still brings about its usual "vagus stuff" effect at postganglionic terminals in the heart when the cardiac vagus has been just completely paralyzed by sodium citrate.

Tests were carried out with five decerebrate cats to find whether the introduction of calcium ions into the circulation just previous to (or along with) the sodium citrate injection would tend to protect the vagus mechanism against paralysis by the sodium citrate (i.e., so that more sodium citrate would then be required to paralyze) as had already been found in case of the turtle. In addition, since sodium citrate of sufficient dosage to paralyze the vagus tended, also, to lower the blood pressure and, finally, to slow the heart, we wished to learn whether the added calcium ions could protect the animals against these effects, as well as against the vagus paralysis. In one experiment calcium gluconate (4 per cent) was used to supply the extra calcium ions, and in four experiments a solution of calcium chloride (0.84 normal) was used. In one of the four experiments (experiment 15), 2.06 cc. per kilogram of this $CaCl_2$ solution was injected into the saphenous vein of a cat in two equal doses administered about eight minutes apart—i.e., a total of 6 cc. of the $CaCl_2$ solution to a female cat weighing 2.9 kgm. The injections were made slowly—the time for each injection of 3 cc. being about one minute. Immediately after the last calcium chloride injection we began the sodium citrate injections. Five injections were made at the rate already described for citrate injections, testing with maximal stimulations after each injection, before the vagus was completely paralyzed. The injections made were 4.8,

2.0, 1.9, 2.0, and 1.7 cc.—a total of 12.4 cc. of the 10 per cent sodium citrate solution. Thirty-five minutes before the calcium chloride injection had been made, it had taken only 6.2 cc. of the sodium citrate solution to completely paralyze the vagus in this same cat.

In case of the other three experiments in which calcium chloride was used, the animals were first bled to the danger point into a measured amount of sodium citrate solution; then the citrated blood was transfused into the same animal from which the blood had been drawn. In two of these three experiments (experiments 19 and 20) we waited for recovery of vagus inhibition after the first transfusion of citrated blood. (This first test transfusion was made to prove that the measured amount of sodium citrate solution used *would* paralyze the vagus and lower blood pressure in that particular animal.) Then the animal was bled a second time into sodium citrate solution, as before. Immediately thereafter, in experiment 19, the calcium chloride (1.1 cc. per kilogram) was injected at the same time the citrated blood was transfused into the saphenous vein. A short rubber tube connected the transfusion burette with the cannula in the saphenous vein; the needle of the syringe containing the calcium chloride solution was simply thrust through the wall of this connecting tube and injection of the extra calcium was thus made into the citrated blood as it flowed into the vein. By this procedure the vagus was entirely protected from any paralyzing effect of the sodium citrate during the second transfusion, and the extra calcium used proved to be sufficient also, to keep the citrate from causing any fall in blood pressure; there was, on the other hand, a steady rise in pressure during the entire period of citrated-blood transfusion in this experiment, and at the end of the transfusion period the general state of the animal was good. In experiment 20, however, an injection of calcium chloride solution (1.46 cc. per kilogram of body weight) was given just before (not during) the second citrated-blood transfusion. In this experiment, the extra calcium itself caused some temporary irregularity in the heart beat, for a short time, before the transfusion was started.

It was evidently too large a dose, for the rate of injection used, in an animal that had been bled so severely. Irregularity in heart beat, as caused by extra calcium in the blood of the cat, has been described by Shafer, Swift, Peterson and Donahue (9). However, the extra calcium, as in the previous experiments, prevented the citrate in the second blood transfusion from paralyzing the vagus and from lowering blood pressure.

In experiment 25 the order was reversed and 3 cc. of 0.84 N $CaCl_2$ solution (i.e., 1.01 cc. per kilogram) was injected into the saphenous vein after the first (instead of after the second) bleeding of the animal, during the early part of the first citrated-blood transfusion. Each bleeding was made into 6.4 cc. of the 10 per cent sodium citrate solution up to a 65 cc. total of the citrate-blood mixture; and the whole amount was transfused in each case. Blood pressure gradually increased during the first transfusion and a test stimulation made immediately after the transfusion, showed that the right vagus had been protected perfectly against paralysis. Toward the last of the second transfusion, however (no extra Ca being introduced) the heart was slowed very markedly; and for a short time after the transfusion had been completed, the right vagus was found to be completely paralyzed. It recovered rapidly, and was then paralyzed again by repeated intravenous injections of sodium citrate solution (i.e., without bleeding), with recovery each time, before the experiment was ended.

In the experiment (experiment 23) in which calcium gluconate was used to supply extra calcium, we injected 1 cc. of the 4 per cent calcium gluconate per kilogram of body weight after the second bleeding of the animal, but we found that amount to be entirely inadequate to protect the animal; blood pressure fell below the danger level and respirations stopped. We did not attempt, further, to find what the protective dose of calcium gluconate would be, because the 0.84 N $CaCl_2$, used at the rate of about 1 cc. per kilogram of body weight, and injected slowly during the early part of a citrated-blood transfusion seemed to be an entirely satisfactory way to provide the extra calcium ions—

giving perfect protection against the temporarily paralyzing effects of the sodium citrate on the cardiac vagus mechanism and on circulatory activity.

We can say that the final impairment in circulatory activity after larger injections of sodium citrate (viz., the decided slowing of the heart beat) was due to a failure of the heart muscle itself, for the following reasons:

1. The slowing of the heart's beat occurred after the vagus was either almost completely, or quite, paralyzed.

2. In case of one experiment in which we had opened the cat's chest (giving artificial respiration) in order to watch the heart as sodium citrate solution was being injected intravenously, we observed, by the time arterial pressure had fallen considerably and the heart began to be slowed, that the chambers of the heart were distending more and more—not being able to empty themselves completely at each beat.

3. In those experiments, with chest intact, in which we took the blood pressure in the superior vena cava by means of a water manometer, we found that the venous pressure always began to show a rise by the time the citrate had caused the arterial pressure to fall decidedly—and especially as the heart began to be slowed (the vagus being paralyzed)—confirming what we saw in the open chest experiment, that the venous return was good and peripheral resistance was not high at that stage.

That is to say, arterial pressure was not failing because the heart lacked oxygenated blood to pump; and the heart was actually working against a lowered resistance; vagus inhibition was either absent or practically so, and the normal Bainbridge reflex should have tended to strengthen and quicken the heart (when the vagi were left intact), but in spite of all this the beat had actually become weaker and slower, therefore the failure must have been in the condition of the heart itself—and brought about by the sodium citrate. This tendency to failure of the heart did not occur, as already stated when extra calcium was injected into the circulation, either immediately before or during the early part of the citrate injection or citrated-blood transfusion. We give concrete example from one experiment. In

experiment 25, after the *second* bleeding, the venous pressure was −5 mm. water pressure (arterial pressure being then 68 mm. on the mercury manometer—having fallen from 152 mm. before the bleeding was started); by the time the citrated blood had been nearly all transfused, and the heart (unprotected by any extra calcium injection) was beginning to be slowed, the venous pressure had risen to +30 mm. (arterial pressure had then fallen to 138 mm.[2]—but had gone up to 142 mm. in the *early* part of the transfusion). On the other hand, at the *first* bleeding, the venous pressure went to −3 mm. water pressure (arterial pressure was then 116 mm. mercury—having been 140 mm. just before the bleeding was started), and just at the completion of the citrated-blood transfusion (3 cc. of 0.84 N $CaCl_2$ having been injected during the early part of the transfusion) the venous pressure came up to +4 mm. water pressure only, while the arterial pressure had made a gradual increase up to 156 mm. mercury pressure—from which it decreased thereafter only slowly to 152 mm. before the second bleeding was started. The vagi were both uncut during the time all the above manipulations were in progress so that the avenue for the Bainbridge reflex was intact when blood pressure at the right auricle went high. Immediately after this first citrated-blood transfusion, when the right vagus was stimulated, the heart was slowed, arterial pressure fell temporarily, and venous pressure (during the stimulation period) went up temporarily from +4 to +22 mm. When the vagus was stimulated immediately after the second citrated-blood transfusion (with no extra calcium) heart rate and venous pressure did not change. The evidence seems clear that sodium citrate brings about its effects, on the vagus mechanism and on the heart muscle, through a lowering of the calcium ions to a critical point. This is now found true for the cat just as had been shown to be true (former citation) for the turtle.

Our smallest "first trial" dose of sodium citrate that caused paralysis of the vagus in any cat was, under ether anesthesia, a dose of 0.091 gram per kilogram of body weight. At the same

[2] Arterial pressure did not fall dangerously low in this case, before the heart was slowed, as it did in some experiments.

rate, for a man weighing 61 kgm. that would amount to a total dose of 5.55 grams. Lewisohn (10) gives 5 grams of sodium citrate in a transfusion as the danger dose for an adult human. These values correspond closely, but the dose needed in any transfusion in man need never go above half that amount. However, we found that those of our cats that had been weakened and had low arterial pressure from bleeding to the danger point were always somewhat more susceptible to collapse from failure of the heart and respiratory center when we gave a sodium citrate transfusion than were animals that had not been bled. We suggest that the immediate collapse which sometimes occurs in case of the human subject, when given a transfusion with citrated blood—especially where the heart is weak (as after injury with great loss of blood)—may be at least in part due to the lowering of calcium ions in the blood (and in the heart) by the sodium citrate. If such is the case, our work indicates that it would be a safety procedure to give a slow injection of the proper amount of calcium chloride (extra calcium ions) during the first part of any citrated-blood transfusion to take care of excess sodium citrate. The dose of calcium chloride would depend on the amount of citrate to be introduced during the transfusion.

SUMMARY

1. Sodium citrate solution may be injected into the blood of a mammal (the cat) in sufficient amount to temporarily completely paralyze the cardiac vagus with gradual but entire recovery in twenty to thirty minutes. Similar or slightly larger doses tend, also, to lower blood pressure, slow the heart, and impair respiratory movements.

2. Using acetylcholine, evidence is found that the sodium citrate paralyzes the cardiac vagus at the synapses between pre- and postganglionic fibers in case of the cat just as was previously found by direct evidence in case of the turtle.

3. Sodium citrate seems to cause the effects (described in no. 1 above) by lowering the calcium ions in the blood and in the heart to the critical point; and all these ill effects may be prevented (in decerebrate animals) by intravenous injection of calcium chloride

solution, in proper amount, either immediately before or during the early part of the sodium citrate transfusion, whether the citrate be given alone or as citrated blood. It is suggested that the immediate "citrate reaction" collapse that sometimes occurs in human subjects during a citrated-blood transfusion might thus be prevented.

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Animal experimental p- and clinical tests of the treatment of cardiac glycoside-induced arrhythmias with sodium citrate

by Wojciech Smolarz, Stanislaw Kosmider and Kazimierz Zajusz)

The problem of glycoside intoxication has aroused increasing interest for some years in view of the increasing use of saliurethic and other K-secreting substances. In the elimination of toxic glycoside effects are used in practice the calcium effect, which is synergistic with regard to cardiac glycosides, and the antagonistic potassium effect. In the therapy potassium is used today orally and intravenously (3,4,8,12) or Na2 EDTA in the continuous drop. (2,5,9). These agents improve, on the one hand, the disturbed potassium gradient, and reduce, on the other hand, the concentration of the biologically effective calcium in the range of the myofibrils. The regulating roll of calcium in the potassium migration into the cell has not yet been sufficiently clarified (1,11).

The present animal experimental-and clinical tests concern variations of the Ca/K quotient in the therapy of glycoside-induced arrhythmias.

Investigated material and technique

The investigations were carried out:

a) on dogs which were intoxicated with cardiac glycosides and then treated with sodium citrate or potassium chloride to eliminate the arrhythmias.

b) on cardiac patients with glycoside induced arrhythmias in the course of a clinical treatment, who were given sodium citrate to eliminate the arrhythmias.

ad a) The investigated cases comprised 18 mongrel dogs or 5-7 kg body weight. The animals were narcotized with pentotal and intoxicated intravenously with 0.15 mg digoxin/kg weight, after a control ecg had been taken.

Usually polymorphic extrasystoles, bigeminy-nodal or ventricular rhythms appeared after a few minutes, which lasted several hours in the control animals. 15 minutes after the intoxication, it was tried to stop the arrythmias by:

1. reducing the calcium level by means of 4% sodium citrate, which was injected intravenously (10 experiments)
2. administering KCl in the continuous drip and adding subsequently sodium citrate (8 experiments)

Apart from the recording of the ecg curves in certain intervals, the potassium- and calcium level were noted before the glycosides were given, after the arrhythmias had been eliminated, and the amount of the dose necessary to eliminate the arrythmias was determined.

ad b) 2 patients who were treated with glycosides for cardiac insufficiency and who showed signs of overdosage in the form of cardiac arrhythmias, received within 1 hour in the intravenous continuous drip 1.2 - 2.0 sodium citrate in physiological salt - solution. Ecg recordings were made before, during and after the sodium citrate application, and the time necessary to eliminate the arrhythmias was recorded. At the same time the calcium level was determined before and after the treatment according to the method by Kovacs (7).

Results of the investigations

a) Animal experimental results

1. Effect of Na-citrate

In all 10 animals the extrasystoles and the bigeminy resp. already disappeared during the application of sodium citrate or immediately thereafter. The change to the sine rhythm could be recorded electrographically in practically all cases (fig. 1). The total dose of sodium citrate was 0.6 to 1.4 g. Only in 2 cases did the elimination of the arrhythmias have a lasting effect; in the other 8 cases the arrhythmias recurred after 5-30 minutes. The calcium level in the serum before the test of 4.8 - 8.5 m Eq/l was lowered by the citrate by 1.0 - 3.5 mg Eq/l. Three animals

received a sublethal glycoside dose. By repeated application of sodium citrate until tetanis spasms appeared, it was possible to cut the arrhythmias and to keep the animals alive. The potassium content in the serum remained unchanged before and after the test.

2. Effects of potassium chloride and Na-citrate

8 animals received KCl in the continuous drip (0.5 g within ½ hour) and then sodium citrate intravenously. In this test series too, we obtained either very good results (fig.2) or ventricular fibrillation appeared suddenly (3 animals, fig.3) This complication can not be accounted for by an increased potassium or reduced calcium-level in the serum. The average dose of sodium citrate was 0.4 g. The values of the potassium- and calcium content in the blood before and after the test showed no significant difference.

Fig. 1 Effect of sodium citrate on glycoside-induced cardiac arrhythmias in doge. a) control curve b) 15 minutes after application of digoxin c) 2 minutes after Na-citrate d) control curve after 20 minutes

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Fig.2 Influence of KCl and Na-citrate on glycoside-induced arrhythmias in dogs. a) control curve b) 15 minutes after application of digoxin; c) 2 minutes after KCl and Na-citrate d) control curve after 60 minutes

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Fig. 3 Ventricular fibrillation after KCl and Na-citrate applications in doge a) control curve b) 15 minutes after application of digoxin c) ventricular fibrillation after KCl and Na-citrate

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Fig.4 Influence of Na-citrate on arrhythmias during digitalis thereapy in man a) bigeminy rhythm; b) after 1.5 g Na-citrate application

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b) Clinical observations

For the treatment with sodium citrate we used those cardiac patients who had shown various arrhythmias in the course of a glycoside therapy. Though a positive relation to the glycoside intoxication could not be established in all cases, it was as a rule patients with cardiac insufficiency

who had taken thiazide diuretics for some time and who therefore showed lower potassium values in the serum. The sensitivity to glycosides was particularly high in these patients. Prior to the therapy we found in these patients in 10 cases the typical arrhythmias in the form of bigeminy rhythm, in the other cases we found ventricular extrasystoles of a heterotopic or of nomotopic form. In some cases the arrhythmias were accompanied by typical dyspeptic symptoms, and further treatment with glycosides was therefore impossible. Sodium citrate was applied intravenously in 5% glucose solution in the continous drip under constant observation of the patients. The dose was 1.2 to 2.0 g. When thearrhythmias subsided, the application was stopped. The infusions were tolerated well by all patients. There were no side effects. The arrhythmias disappeared in all treated patients during or after the infusion, which could be verified electrographically (fig.4).

If arrhythmias appeared again, which was the case in the majority of the patients, the infusion was repeated with smaller amounts of sodium citrate until the rhythm was back to normal. The subjective feeling of the patients improved as a rule after normalization of the arrhythmias. The calcium level in the serum before the application of sodium citrate was 4.6 - 5.9 m Eq/l, and dropped after the infusion by 0.3 - 0.5 m Eq/l.

Discussion

There are various possibilities of eliminating glycoside-induced arrhythmias. The principle of each method is based on a variation of the concentration ratios of calcium and potassium-ions (6). Lowering of the calcium level by the application of sodium citrate proved to be a successful and harmless method. In all cases it was possible to eliminate arrhythmias at least for a short time. Up to now Na2 EDTA had been used at the Clinic for this purpose. But with these substances certain side effects are observed which prevent the wide use of this method. Particularly histamine-like symptoms, a great hypotonic effect and local irritations of the veins were observed. According to our experience so far, there are no principal

contra-indications for the introduction of sodium citrate as a means of reducing the calcium level into the clinical practice. Sodium citrate as an addition to preserved blood is used daily in man. A detailed description of the behavior of sodium citrate in the intravenous injection in animals and in man is found in Ludbrook and Wyman (10). According to the experiences of these authors, sodium citrate should be avoided in liver- and kidney damages, as well as in hypothermy.

In the patients observed by us the results of the therapy with sodium citrate were good, and no side effetts were observed. On the basis of our tests, this method is suitable to eliminate life-endangering arrhythmias in the course of a glycoside treatment. The positive result in all cases indicates that sodium citrate can also eliminate arrhythmias of other genesis. But tests on a larger group of patients would have to be made in this respect, because they have today practical aspects.

The coupling of the supply of potassium-ions with subsequent reduction of the calcium-ions by sodium citrate permitted a normalization of the rhythm with substantially smaller doses of this substance. The sudden ventricular death of three animals indicates, however, that rapid and disadvantageous variations of the Ca/K-ion ratio may occur. These changes are too short to be recorded with the conventional potassium- and calcium determination methods. For this reason we consider this method of treating digitalis intoxication dangerous.

Conclusions

1. Sodium citrate is suitable to eliminate glycoside-induced cardiac arrhythmias in dog.

2. The application of potassium-ions and sodium citrate in digitalis intoxication of dogs is considered dangerous because of the rapid change in the ion ratio.

3. The treatment of glycoside-induced cardiac arrhythmias with sodium citrate is considered a safe and successful method on the basis of our own clinical observations.

Summaries in German and English.

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(Translated by Carl Demrick Associates,Inc/IE/t)

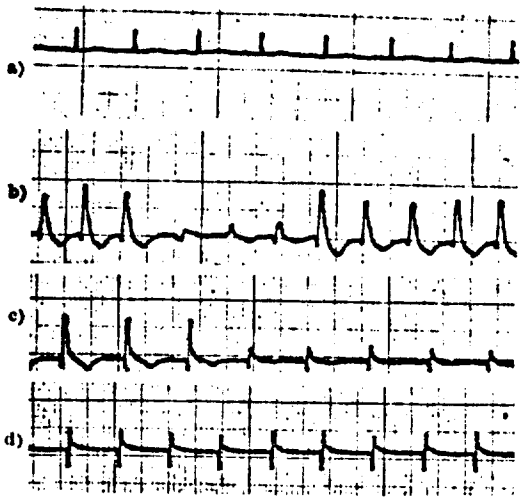

Figure 1

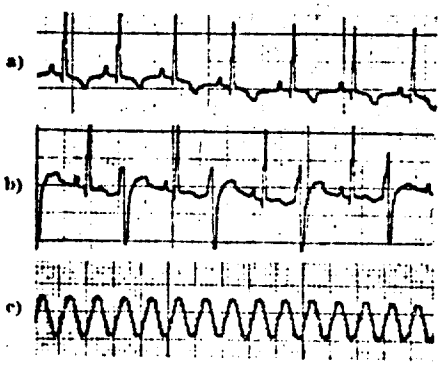

Figure 2

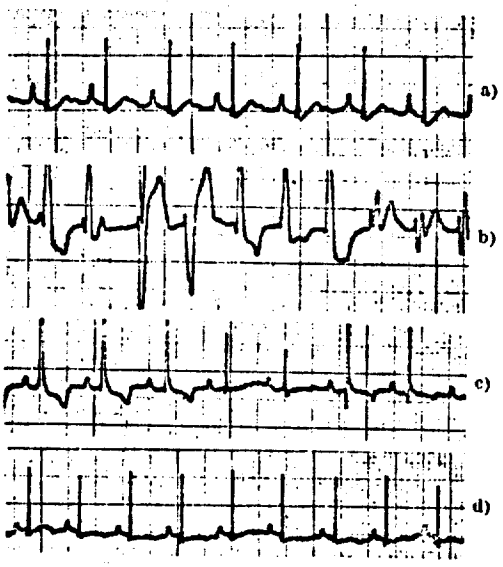

Figure 3

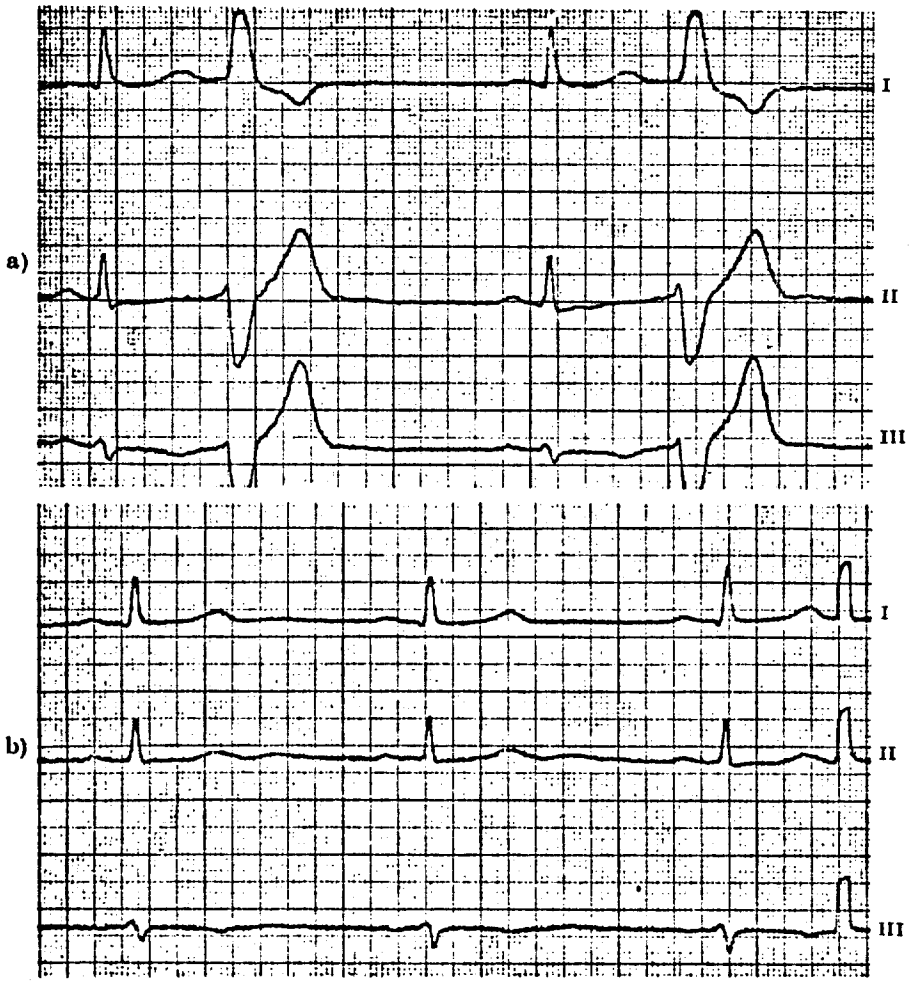

Figure 4

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Tierexperimentelle und klinische Versuche einer Behandlung von herzglykosidbedingten Arrhythmien mit Natrium-Zitrat

Von Wojciech Smolarz, Stanisław Kośmider und Kazimierz Zajusz

Mit 4 Abbildungen in 13 Einzeldarstellungen

(Eingegangen am 15. November 1965)

Das Problem der Glykosidintoxikation ruft seit einigen Jahren angesichts des wachsenden Gebrauchs saliuretischer und anderer K-ausscheidender Mittel steigendes Interesse hervor. Bei der Beseitigung toxischer Glykosidwirkungen findet die den Herzglykosiden gegenüber synergistische Kalzium- und antagonistische Kaliumwirkung praktische Anwendung. In der Therapie verwendet man heute Kalium per os und intravenös (3, 4, 8, 12) oder Na_2 EDTA im Dauertropf (2, 5, 9). Diese Mittel verbessern einerseits den gestörten Kaliumgradienten, vermindern anderseits die Konzentration des biologisch wirksamen Kalziums im Bereich der Myofibrillen. Die regulierende Rolle des Kalziums bei der Kaliumwanderung in die Zelle ist bisher nicht genügend geklärt (1, 11).

Vorliegende tierexperimentelle und klinische Untersuchungen betreffen Veränderungen des Ca/K-Quotienten bei der Therapie glykosidbedingter Arrhythmien.

Untersuchungsmaterial und Methodik

Die Untersuchungen wurden durchgeführt:

a) an Hunden, welche mit Herzglykosiden intoxiert und danach mit Natriumzitrat oder Kaliumchlorid zwecks Beseitigung der Arrhythmien behandelt wurden.

b) an Herzkranken mit glykosidbedingten Arrhythmien im Verlauf einer klinischen Behandlung, welchen Natriumzitrat zwecks Beseitigung der Arrhythmien verabreicht wurde.

Ad a) Das Untersuchungsmaterial bestand aus 18 mischrassigen Hunden mit 5–7 kg Körpergewicht. Die Tiere wurden mit Pentotal narkotisiert und nach Ausführung eines Kontroll-Ekg mit 0,15 mg/kg-Körpergewicht Digoxin i.v. intoxiert.

Gewöhnlich traten nach einigen Minuten polymorphe Extrasystolen, Bigeminus-Nodal- oder Kammer-Rhythmus auf, die bei Kontrolltieren einige Stunden andauerten. 15 Minuten nach der Intoxikation wurde der Versuch unternommen, die Arrhythmien zu beseitigen durch:

1. Erniedrigung des Kalziumspiegels mit Hilfe von 4% Natriumzitrat, das intravenös verabreicht wurde (10 Experimente).
2. Verabreichung von KCl im Dauertropf und anschließende Natriumzitrat-Zugabe (8 Experimente).

Außer der Registrierung der Ekg-Kurven in bestimmten Abständen wurden der Kalium- und Kalziumspiegel vor Verabreichung der Glykoside und nach Beseitigung der Arrhythmien festgestellt und die zur Beseitigung der Arrhythmien nötige Dosis des Mittels bestimmt.

Ad b) 21 Patienten die wegen Herzinsuffizienz mit Glykosiden behandelt wurden und Zeichen von Überdosierung in Form von Herzrhythmusstörungen aufwiesen, erhielten innerhalb einer Stunde im intravenösen Dauertropf 1,2–2,0 Natriumzitrat in physiologischer Kochsalzlösung. Ekg-Aufzeichnungen wurden vor, während und nach der Natriumzitrat-Verabreichung vorgenommen und die zur Beseitigung der Arrhythmien erforderliche Zeit festgehalten. Gleichzeitig wurde auch der Kalziumspiegel nach der Methode von *Kovacs* (7) vor und nach der Behandlung bestimmt.

Untersuchungsergebnisse

a) Tierexperimentelle Resultate

1. Effekte von Na-Zitrat

Bei allen 10 Tieren gingen die Extrasystolen bzw. der Bigeminus bereits während der Verabreichung von Natriumzitrat oder aber unmittelbar danach zurück. Der Übergang zum Sinusrhythmus ließ sich in fast allen Fällen elektrokardiographisch registrieren (Abb. 1). Die Gesamtdosis des verabreichten Natriumzitrats betrug 0,6 bis 1,4 g. Nur in 2 Fällen war die Beseitigung der Rhythmusstörungen von Dauer. In den übrigen 8 Fällen kehrten nach 5–30 Minuten die Arrhythmien wieder. Der Kalziumspiegel im Serum vor dem Versuch von 4,8–8,5 m Eq/l wurde

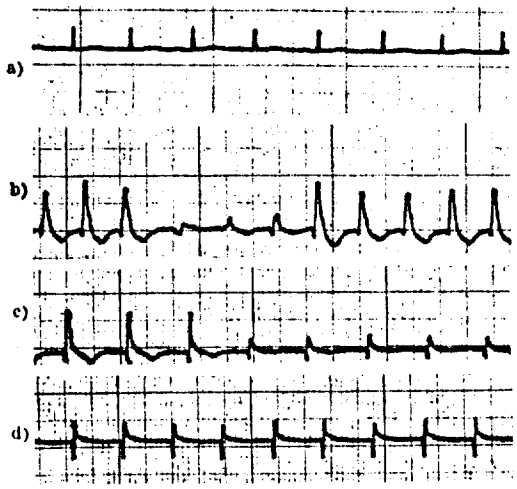

Abb. 1. Wirkung von Natriumzitrat auf glykosidbedingte Herzarrhytmien bei Hunden. a) Kontrollkurve, b) 15 Min. nach Digoxinverabreichung, c) 2 Min. nach Na-zitrat, d) Kontrollkurve nach 20 Min.

durch das Zitrat um 1,0–3,5 m Eq/l gesenkt. Drei Tiere erhielten eine subletale Glykosiddosis. Durch wiederholte Verabreichung von Natriumzitrat bis zum Auftreten tetanischer Krämpfe gelang es die Rhythmusstörungen zu koupieren und die Tiere am Leben zu erhalten. Der Kaliumgehalt im Serum vor und nach dem Versuch blieb unverändert.

2. Effekte von Kaliumchlorid und Na-Zitrat

8 Tieren wurde KCl im Dauertropf (0,5 g innerhalb ½ Stunde) und danach Natriumzitrat i.v. verabreicht. Auch in dieser Versuchsreihe wurden entweder sehr gute Ergebnisse erzielt (Abb. 2), oder aber es kam zu

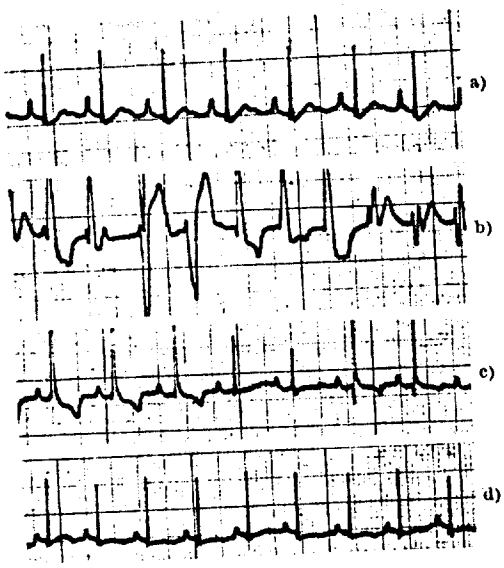

Abb. 2. Einfluß von KCl und Na-zitrat auf glykosidbedingte Rhythmusstörungen bei Hunden. a) Kontrollkurve, b) 15 Min. nach Digoxinverabreichung, c) 2 Min. nach KCl und Na-zitrat, d) Kontrollkurve nach 60 Min.

plötzlichem Kammerflattern (3 Tiere Abb. 3). Diese Komplikation ließ sich nicht durch erhöhten Kalium- oder erniedrigten Kalziumspiegel im Serum erklären. Die durchschnittliche Dosis des verabreichten Natriumzitrat betrug 0,4 g. Die Werte des Kalium- und Kalziumgehaltes im Blut vor und nach dem Versuch wiesen keinen signifikanten Unterschied auf.

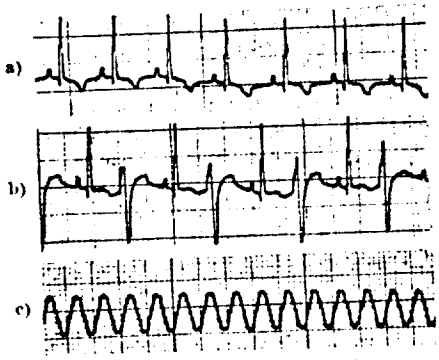

Abb. 3. Kammerflattern nach KCl und Na-zitrat-Verabreichung bei Hunden. a) Kontrollkurve, b) 15 Min. nach Digoxinverabreichung, c) Kammerflattern nach KCl und Na-zitrat.

b) Klinische Beobachtungen

In der Krankengruppe wurden zur Behandlung mit Natriumzitrat solche Herzkranke herangezogen, welche im Verlauf einer Glykosidtherapie verschiedene Rhythmusstörungen aufwiesen. Zwar konnte nicht in jedem Fall ein sicherer Zusammenhang mit der Glykosidvergiftung bewiesen werden, in der Regel waren es aber Kranke mit Herzinsuffizienz, die längere Zeit Thiazid-diuretika eingenommen hatten und deshalb niedrige Kaliumwerte im Serum aufwiesen. Die Empfindlichkeit gegenüber Glykosiden war bei ihnen also besonders hoch. Vor der Therapie fanden wir bei diesen Kranken in 10 Fällen die typischen Rhythmusstörungen in Form von Bigeminus-Rhythmus, in den übrigen Fällen waren es Kammer-Extrasystolen, heterotoper oder nomotoper Form. In einigen Fällen

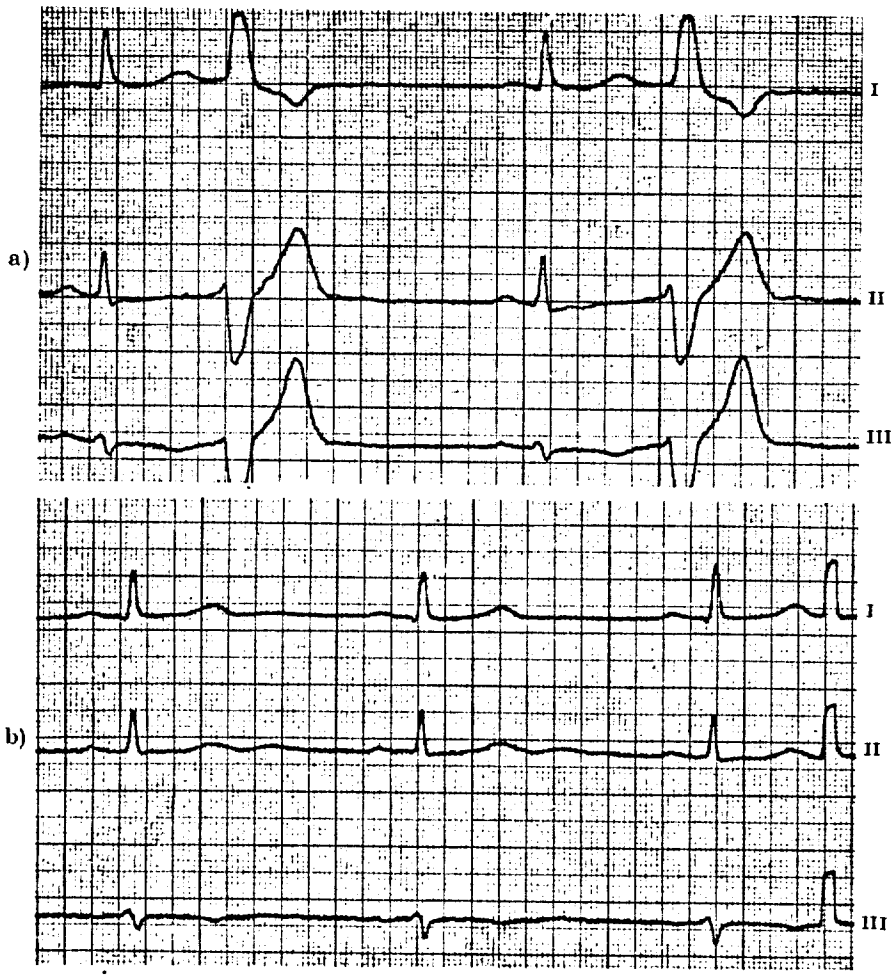

Abb. 4. Einfluß von Na-zitrat auf Rhythmusstörungen während Digitalistherapie bei Menschen. a) Bigeminus-Rhythmus, b) nach 1,5 g Na-zitrat-Verabreichung.

waren die Rhythmusstörungen mit typischen dyspeptischen Erscheinungen verbunden und eine weitere Behandlung mit Glykosiden wurde dadurch unmöglich. Natriumzitrat wurde in 5 % Glukoselösung im intravenösen Dauertropf unter ständiger Beobachtung der Patienten verabreicht. Die Dosis betrug 1,2 bis 2,0 g. Bei Zurücktreten der Rhythmusstörungen wurde die Verabreichung unterbrochen. Die Infusionen wurden von allen Patienten gut vertragen. Es traten keinerlei Nebenerscheinungen auf. Die Rhythmusstörungen gingen bei allen behandelten Patienten während oder nach der Infusion zurück, was elektrokardiographisch bestätigt werden konnte (Abb. 4).

Wenn es zu erneuten Rhythmusstörungen kam, was bei der Großzahl der Behandelten der Fall war, wurde die Infusion mit kleineren Mengen Natriumzitrat bis zum Rhythmusausgleich wiederholt. Das subjektive Befinden der Patienten verbesserte sich in der Regel nach Normalisierung der Arrhythmien. Der Kalziumspiegel im Serum betrug vor der Verabreichung von Natrium citricum 4,6–5,9 m Eq/l und verminderte sich nach der Infusion um 0,3–0,5 m Eq/l.

Diskussion

Es bestehen verschiedene Möglichkeiten glykosidbedingte Arrhythmien zu beseitigen. Das Prinzip jeder Methode beruht auf einer Veränderung des Konzentrationsverhältnisses von Kalzium- zu Kaliumionen (6). Die Senkung des Kalziumspiegels durch Verabreichung von Natriumzitrat hat sich als erfolgreiche und gefahrlose Methode erwiesen. In allen Fällen gelang es, die Rhythmusstörungen zumindest für kurze Zeit zu beseitigen. Bisher wird in der Klinik zu diesem Zweck Na_2 EDTA angewandt. Bei diesem Mittel sind jedoch einige Nebenerscheinungen zu beobachten, welche die breite Anwendung dieser Methode verhindern. Es wurden vor allem histaminähnliche Erscheinungen, eine stark hypotonische Wirkung, und lokale Venenreizungen beobachtet. Nach unseren bisherigen Erfahrungen bestehen keine grundsätzlichen Kontraindikationen einer Einführung von Natriumzitrat als Mittel zur Senkung des Kalziumspiegels in die Klinik. Natriumzitrat als Zusatz zu Blutkonserven wird bei Menschen täglich angewandt. Eine genaue Beschreibung des Verhaltens von Natriumzitrat bei intravenöser Verabreichung bei Tieren und Menschen geben *Ludbrook* und *Wyman* (10). Den Erfahrungen dieser Autoren gemäß soll Natriumzitrat bei Leber- und Nierenschäden sowie bei Hypothermie vermieden werden.

Bei dem von uns beobachteten Krankengut waren die Erfolge der Therapie mit Natriumzitrat gut, und es wurden keine Nebenerscheinungen beobachtet. Auf Grund unserer Untersuchungen ist diese Methode geeignet, lebensbedrohliche Arrhythmien im Verlauf einer Glykosidbehandlung zu beseitigen. Der sichere Erfolg in allen Fällen deutet darauf hin, daß Natriumzitrat auch Arrhythmien anderer Genese zu beseitigen vermag. Untersuchungen in dieser Richtung müßten aber in größeren Krankengruppen durchgeführt werden, weil sie heute praktische Aspekte besitzen.

Die Koppelung einer Zuführung von Kaliumionen mit anschließender Verminderung der Kalziumionen durch Natriumzitrat ermöglichte eine Rhythmusnormalisierung bei wesentlich kleineren Mengen dieses Mittels. Der plötzliche Kammertod dreier Tiere zeugt aber immerhin davon, daß es zu raschen und unvorteilhaften Veränderungen des Ca/K-Ionen-Verhältnisses kommen kann. Diese Veränderungen sind wohl zu kurzfristig um mit den herkömmlichen Methoden der Kalium- und Kalziumbestimmung festgehalten werden zu können. Deshalb betrachten wir diese Behandlungsmethode der Digitalisintoxikation als gefährlich.

Folgerungen

1. Natriumzitrat eignet sich zur Beseitigung glykosidbedingter Herzrhythmusstörungen bei Hunden.
2. Eine Verabreichung von Kaliumionen und Natriumzitrat bei Digitalisintoxikation von Hunden wird wegen rascher Veränderung des Ionenverhältnisses als gefährlich betrachtet.
3. Die Behandlung glykosidbedingter Herzrhythmusstörungen mit Natriumzitrat wird auf Grund eigener klinischer Beobachtungen als gefahrlose und erfolgreiche Methode angesehen.

Zusammenfassung

18 Hunde wurden mit Digoxin intoxiert um Arrhythmien hervorzurufen. Darauf versuchte man, die Rhythmusstörungen durch Verabreichung von Natriumzitrat bzw. Verabreichung von Kaliumchlorid und Natriumzitrat zu beseitigen. Die besten Ergebnisse bei der Rhythmusausgleichung wurden mit Natriumzitrat allein erzielt. Die Applikation beider Mittel führte bei wesentlich kleinerer Dosis zwar ebenfalls zu positiven Resultaten. In einigen Fällen jedoch trat dabei plötzliches Kammerflattern ein.

Intravenöse Verabreichung von Natriumzitrat bei Herzkranken mit Rhythmusstörungen hat sich als erfolgreiche und gefahrlose Behandlungsmethode erwiesen. Unter Einfluß von Natriumzitrat kam es bei allen Patienten zur Rhythmusnormalisierung. Die Beseitigung der Rhythmusstörungen war jedoch nicht von Dauer und bedurfte Wiederholungen der Infusionen in kleineren Mengen. Mit dieser Methode können akute Digitalisintoxikationen in der Klinik erfolgreich behandelt werden.

Summary

Experimental and clinical studies in the treatment of arrhythmias due to digitalis by natrium citrate

18 dogs were intoxicated with digoxin for inducing cardiac arrhythmias. After that, the attempt was made to remove the arrhythmias by injections of Na-citrate or KCl and Na-citrate together. The best results were obtained giving Na-citrate alone. By application of the two compounds an essential lower dosis was necessary to obtain positive results. In a few cases, however, ventricular flutter appeared.

Na-citrate given intravenously to patients with cardiac arrhythmias due to digitalis has proved as a successful and undangerous method. Normalization of rhythm was obtained in all patients observed, but infusions of lower amounts of Na-citrate must have been repeated because of remissions of rhythm disturbances.

This method can be sucessfully used in treatment of acute digitalis toxicity often observed in clinical practice.

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(Direktor: Prof. Dr. F. Grosse-Brockhoff)

Formale und zeitliche Beziehungen zwischen Oesophagoatriogramm und Ultraschallkardiogramm

Von S. Effert, L. Sachs und W. Bleifeld

Mit 4 Abbildungen

(Eingegangen am 22. November 1965)

Mit Applikation des elektroakustischen Wandlers über dem 3. bzw. 4. ICR links parasternal läßt sich im Ultraschallkardiogramm eine Kurve registrieren, die die übliche venöse Grundcharakteristik aufweist, mit 2 Maxima, denen jeweils ein Minimum folgt. Bei Mistralstenose ist die Kurve in charakteristischer Weise verändert. Das Oesophagoatriogramm, registriert mit der Methode nach *Friese* (3), zeigt einen grundsätzlich analogen Kurvenverlauf. Beide Methoden geben über die hämodynamischen Verhältnisse im Bereich des linken Vorhofes Aufschluß. Für die Ultraschallkardiographie ist neuerdings gezeigt worden (1, 2), daß die Bewegung des Klappenapparates selbst mit diesem Verfahren aufgezeichnet wird. Demgegenüber wird mit der Oesophagoatriographie die Bewegung der Hinterwand des linken Vorhofes im Oesophagoatriogramm graphisch registriert. Vorhofdruckkurve und Oesophagoatriogramm verlaufen nach den Untersuchungen von *Loogen* und *Sachs* sowie *Ockenga* und Mitarb. weitgehend parallel. Es liegt damit auf der Hand, zu untersuchen, welche zeitlichen und formalen Beziehungen zwischen Oesophagoatriogramm und Ultraschallkardiogramm bestehen.