(1950) found no change in the ascorbic acid content of chick adrenals even though corticosteroids were released.

While our experiments were in progress Nadel and Schneider (1951) reported that the "formaldehydogenic substances" in guinea-pig urine showed a five-fold increase in the later stages of scurvy.

A.C.T.H. can produce a fall in the level of circulating eosinophils in scorbutic animals, as shown by Eisenstein and Shank (1951). From this, and the fact that scurvy is accompanied by adrenal hypertrophy, they concluded that ascorbic acid was not essential for adrenal function and not directly concerned in the formation and release of 11-oxysteroids.

Our experiments seem to confirm an increase of adrenal cortical activity in scurvy as judged by adrenal hypertrophy, and an increase of cortical hormone from the increase of 17-ketosteroid excretion. Both of these changes are reversible by cortisone, although this has no effect on the disease. A.C.T.H. is capable of causing additional hypertrophy. The 17-ketosteroid excretion is certainly increased during the earlier stages of the development of scurvy, but in the fully scorbutic animal, even in the absence of exogenous A.C.T.H., it appears to be nearly maximal. A much larger number of observations must be made before it is definitely established whether the output of these substances is significantly increased under these conditions by A.C.T.H. The balance of evidence favours the view that adrenal cortical activity is increased in scurvy, and can be further increased, in the absence of vitamin C, by A.C.T.H.

Summary

Guinea-pigs placed on a scorbutic diet showed a gradual well-marked increase of varying extent in urinary 17-ketosteroid excretion. This reached a peak in the terminal phases.

Daily administration of A.C.T.H. and cortisone failed to influence the fall in body weight and subsequent death of the animals on a scorbutic diet. Such animals showed similar manifestations of scurvy as untreated scorbutic controls.

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PREPARATION OF CLEAN CHEMICAL SOLUTIONS

WITH SPECIAL REFERENCE TO RADIOACTIVE PHOSPHORUS SOLUTIONS FOR CLINICAL USE

BY

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In the application of radioactive isotopes to biological studies the preparation of clean solutions is a matter of considerable importance Especially is this the case in the medical use of radioactive isotopes, for, in addition to the possible unsuspected localization of the particulate material after injection (see the following article by Lamerton and Harriss), there is the further complication of the uncertainty of the dosage delivered to a particular organ. Indeed, this very effect may account for some of the wide variations in the dose required to produce a given biological change reported in the literature—variations which are commonly attributed to differences in the biological sensitivity of a particular organ in different individuals. There is also the obvious physical complication that the realization of standard solutions of a radioactive isotope is greatly complicated if part of the activity is in a non-exchangeable, particulate form.

The present experiments were carried out on radioactive phosphate solutions as prepared at Harwell for clinical use. It should be noted, however, that the methods employed are of general application and are likely to be effective in all cases in which a high degree of cleanliness of a preparation is of importance.

In the dispensing of radioactive phosphate solutions for clinical use the inactive carrier is isotonic saline (0.9 g. NaCl %) buffered to *p*H 7 with sodium phosphate containing 1 mg. P/ml. A small aliquot of carrier-free radioactive phosphoric acid is then added to a measured volume of the buffered saline in a "pyrex" ampoule, to give an activity of about 1 mc./ml. As the phosphorus content of the mixture is practically the same as that of the carrier, the ratio of P^{32}/P^{31} in this solution is about 3×10^{-6}. The ampoule is sealed and then autoclaved at 20 lb./sq. in. (1.4 kg./cm.^{2}) pressure (temperature 126° C.) for 20 minutes. Specimens of phosphate solution prepared by the methods in use to the end of 1950 were faintly hazy, and on centrifugation at 2,500 r.p.m. for 15 minutes up to 50% of the activity could be removed in the centrifugate. When

freshly distilled water was used to prepare the phosphate solution it was still far from clear. This result prompted a complete overhaul of the dispensing procedure, including the state of cleanliness of the glassware and the preparation of the buffered saline.

The fact that a considerable fraction of the activity is removed with the centrifugate suggests that the latter has taken up the phosphate in a non-exchangeable form. This result could be produced by bacterial contamination, by the presence of inorganic particulate matter, or by the presence of organic substances which can lead to aggregation of phosphate in a non-exchangeable form. At the moment we do not know the relative importance of each of these factors in the present case. It is assumed that the P^{32} is not specifically absorbed, but experiments to check this are to be made.

Testing and Cleaning of the Solutions

It was decided, therefore, that in the preparation of the phosphate solutions precautions should be taken to minimize (*a*) bacterial contamination, (*b*) particulate matter, and (*c*) organic substances.

From the point of view of each of these three factors it is important that all glassware should be specially cleaned immediately before use, avoiding organic cleaning agents, and that in making up the solutions only distilled water drawn directly from the still should be used.

To test the solutions for particulate matter sealed ampoules were examined by transverse illumination. For this purpose an incandescent lamp, fitted in a metal box with converging lens and diaphragm, was used as a light source to give a narrow beam which was made to traverse the solution. The apparatus was enclosed in a dark-box or placed in a dark-room and the particulate matter was then viewed at an angle to the beam, using a low-powered microscope or simple lens. This familiar method of transverse illumination, while giving a somewhat severe test of particulate contamination—since all particles, even those of submicroscopic size, can produce images—was found with suitable comparative standards to give a simple and reliable qualitative test of the general cleanliness of a solution. An important advantage of this method of examination is that there is no need to open the pyrex ampoule.

When sealed ampoules containing radioactive phosphate solutions prepared before the introduction of the special precautions were examined in this way they showed considerable particulation, the particles varying from those settling fairly rapidly to those showing Brownian movement.

With ordinary chemical methods of cleaning the glass, it was found that considerable particulate contamination was observed even with samples of distilled water drawn straight from the still, and it appeared that such methods were quite ineffective for removing dust and siliceous matter adhering to the interior of the ampoule. This result can readily be verified if a glass tube cleaned with nitric acid and then washed freely in distilled water is allowed to drain. Particulate matter adhering to the walls is immediately obvious. This result led to the following method of cleaning pyrex ampoules before filling with the radioactive solution. It is recommended in all cases where clean dust-free glassware is essential and, as is often the case, it is impossible to introduce a mechanical aid such as a brush or scrubber.

Method of Cleaning Pyrex Glass Ampoules

For the reasons already stated the use of detergents was avoided. A few grammes of powdered glass were inserted into the ampoule and concentrated nitric acid was added. The interior of the glassware was then thoroughly washed with this mixture, using a rapid rotary motion to ensure that full mechanical action of the powdered glass was obtained. The acid mixture was warmed to about 50° C. and then poured off and the container thoroughly washed out with tap-water fed into the inverted ampoule by means of a narrow glass tube for three to four minutes. This method of washing in an inverted position by means of a continuous jet of water is important. With the ampoule still inverted it was washed for a similar time with a jet of distilled water drawn freshly from the still. Finally, the ampoule was rinsed three times with water running direct from the still and then partly filled with this water and closed with a rubber cap.

The ampoule was examined by transverse illumination. A successful cleansing resulted in practically no particulate material being observed on transverse illumination—that is, a uniformly dark ground. Such an ampoule was regarded as clean, and after emptying out the water was sealed with a rubber cap for immediate use.

It is interesting to note that other types of glassware, including soda-glass and "phoenix" glass, when cleaned in this way and filled with freshly distilled water showed no appreciable particulate contamination.

Preparation of Inactive Phosphate Buffer and Isotonic Saline

In order to examine the particulate contamination introduced by the saline and the phosphate buffer in initial experiments the two solutions were prepared separately.

Freshly prepared analar salt solutions made up in clean glassware with distilled water drawn straight from the still always showed some particulate contamination, due, no doubt, to foreign insoluble material in the analar salts. To overcome this difficulty the following procedure was adopted.

The saline and buffer phosphate solutions were transferred to clean flasks and separately refluxed for 30 minutes to facilitate coagulation of insoluble material. The cooled solutions were then poured into clean centrifuge tubes, which were closed with a flexible rubber cap and spun at 2,500 r.p.m. in a large centrifuge (arm radius, 13 cm.) for 15 minutes. The upper half of the supernatant was then carefully siphoned off through a clean narrow-bore tube into a clean spherical flask. This procedure was found to give solutions which on transverse illumination were noticeably superior to the aqueous solutions prepared by dissolving the salts directly.

In the case of the phosphate buffer solutions which were prepared from analar disodium and mono-sodium phosphates, even after centrifugation it was found that the solutions on examination by transverse illumination always showed an opalescence due to a high concentration of very small particles. The possible contaminants in the analar salt suggested that this suspension might be due to an insoluble phosphate, but upon acidifying the solution the degree of opalescence was

unchanged. Attempts to obtain solutions free from these small particles were without success, and accordingly we abandoned the use of analar phosphates in favour of the following preparation in which "specpure" Na_2CO_3 was dissolved in analar orthophosphoric acid. 0.29 g. of the "specpure" carbonate was dissolved in distilled water drawn direct from the still. To this 0.32 g. of analar orthophosphoric acid was slowly added and the solution made up to 100 ml., so giving a phosphate buffer of *p*H 7 containing 1 mg. P per ml.; 0.9 g. NaCl was then added The resulting solution was then refluxed and centrifuged as before. The degree of opalescence was less than in the previous case, and this procedure gave the cleanest buffered saline solution obtained.

The whole procedure was carried out in a still atmosphere as free from dust as possible. A measured small volume of phosphoric acid containing P^{32} was added to a suitable aliquot of the buffered saline solution in a clean ampoule, and the latter was then sealed and autoclaved as soon as possible. It is to be noted that the method of preparation described reduces the possibility of bacterial infection to a minimum while avoiding the rather serious complication of total aseptic conditions.

Results

The ampoule was opened and the β-particle activity of the uniformly mixed solution was compared with that of the supernatant solution after centrifuging at 2,500 r.p.m. for 20 minutes. This comparison was made with a standard "M6" liquid counter and also with differential β-particle ionization chambers, whereby the difference between the activity of the uniform solution and the supernatant was measured directly.

The results of tests on five different samples are tabulated below.

Sample No.	Ratio of Activity/ml. of Uniformly Mixed Solution to that of the Supernatant
X1	1·017
X2	1·015
+3	1·011
+4	1·012
+5	1·015

X, by counting technique. +, by ionization chambers.

It is concluded that the difference between the activity of the uniformly mixed solutions and the supernatant when the present procedure is carried out can be reduced to less than 2%.

It is not clear how far the results would be affected by changes in the carrier content of the solution. This important point is to be investigated.

The World Health Organization reports that the incidence of poliomyelitis was higher in Australia, the Belgian Congo, and the Netherlands than in the corresponding weeks in 1949 and 1950. In Switzerland, too, the incidence was higher; in the United States there were more cases than in 1950 but less than in the previous year. Austria, Canada, and the Western sectors of Berlin also had more cases than in the two preceding years. Scotland, Northern Ireland, and the German Federal Republic on the other hand reported fewer cases this year than in 1950 but more than in 1949; in France also the situation has improved since 1950, and England and Wales had fewer notifications than during the two preceding years.

SOME AUTORADIOGRAPHIC STUDIES OF NON-UNIFORM DISTRIBUTION OF RADIOACTIVE PHOSPHORUS IN TISSUES

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[WITH SPECIAL PLATE]

For the purpose of estimating the dose of radiation received by tissues after the administration of radioactive isotopes, it is generally assumed that there is a uniform, or at least a continuous, distribution of isotope within a particular tissue or part of tissue considered. From the cellular point of view it is unlikely that this assumption will ever be justified, and even from the macroscopic view it is well known that the distribution of isotopes is often by no means uniform, as for instance in the case of iodine in the thyroid. There is clearly a need for a theoretical treatment of what might be called the "micro-dosimetry" of radioactive isotopes, but there is also need for further facts about the non-uniform distribution of isotopes in various tissues when administered in various ways, and for this reason the following observations on the non-uniform distribution of P^{32} in certain tissues are recorded.

Distribution of P^{32} in Rat Tissues Following Intravenous and Intraperitoneal Injection

It was found that certain autoradiographs of sections of rat liver and spleen following intravenous injection of P^{32} (as Na_2HPO_4) in normal saline prepared prior to the introduction of the special precautions described by Harrison and Raymond on page 930 showed by no means a uniform distribution of the isotope. This fact was first observed with contact autoradiographs of sections on *x*-ray film, and examples of such autoradiographs of rat-liver sections when the animals were killed at different times after injection (with 100 to 200 μc. of P^{32}) are shown in Figs. 1A, 2A, and 3A (Special Plate). Similar appearances on the autoradiographs were found in liver sections of rats sacrificed as long as three weeks after injection. The autoradiographs of spleen tissue also showed a similar "spotty" appearance, as did lung and bone marrow. Autoradiographs of other tissues did not show these local high concentrations of isotope. When, however, the injection was given intraperitoneally, no such spotty distribution of the isotope was evident. An example of an autoradiograph of a rat-liver section following intraperitoneal injection of the phosphate is given in Fig. 4.

Preparation of Tissues for Autoradiography

The fixative used for the tissues giving the autoradiographs of Figs. 1, 2, and 3 was 10% formaldehyde. 10-μ sections were cut, floated from water on to slides, and then clamped against "ilfex" *x*-ray film in a printing-frame for the required exposure time. Various investigations were carried out to ensure that the appearances in these autoradiographs were not artifacts.