

THE INORGANIC ELEMENTS IN THE NUTRITION OF THE RAT

In this laboratory it has for years been our practice to use in most of our experimental rations the usual yeast-casein-dextrin-butterfat mixture with the inorganic requirements provided by either salt mixture No. 185 or No. 51. The latter is simpler to prepare than No. 185 and gives excellent results when fed at 6.1 per cent.

Since 1933 we have been observing the results of lowering one or more of the inorganic elements on growth, reproduction and longevity. A preliminary report of our findings was presented at the Fifteenth International Physiological Congress in Moscow in 1935.

Subsequent investigations have revealed striking ab-

normalities in kidney structure. Of 152 animals examined grossly 70 showed hypertrophy, abnormal color and marked pitting of the surface. In several cases there were watery cysts of varying sizes on the surface of the kidneys. In all cases the capsule was easily stripped. Animals on the breeding stock ration of the same age seldom reveal such abnormalities and if so never to so marked an extent.

The nature of these changes is being studied by a pathologist and will be reported in detail at a later date along with the composition of the diets employed.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN APPARATUS FOR THE MICRODETERMINATION OF CHOLESTEROL IN BLOOD

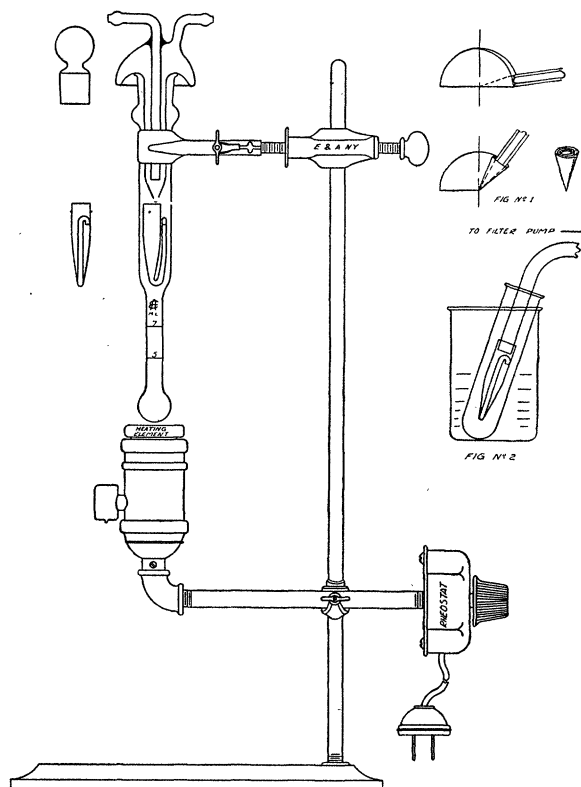
CHOLESTEROL is usually determined by adding acetic anhydride to a chloroform extract and comparing the color with that obtained from a standard solution. A very convenient apparatus for extracting the cholesterol and developing the color without transfer consists of an extraction tube, in the form of the usual Folin-Wu sugar tube, graduated at 5 and 7 cc and furnished with a glass stopper, a micro Soxhlet extractor, designed to fit within it, and a "mushroom" condenser.

The extractor also serves to dry the blood. A disk of filter paper, 5.5 cm in diameter, is folded in half. The pointed end of a pencil is placed between the two halves just at the folded edge, and then the paper is rolled into a narrow cone around the pencil (Fig. 1). This cone of paper is inserted into the wide portion of the extractor well into the taper. 0.2 cc of blood (which can be secured by finger prick¹) are drawn up into a fine tipped pipette and then allowed to soak into the paper. The blood is dried *in situ* by connecting the extractor to a suction pump by means of a heavy-walled rubber tube that fits within the wide end snugly, and then placing the extractor in a dry test-tube immersed in boiling water (Fig. 2). A current of air is drawn through the extractor for fifteen minutes to dry the blood.

The bulb of the extraction tube is filled with dry chloroform, and then the extractor and condenser are placed in it. The extractor is conveniently inserted and removed by inserting a forceps with the jaws closed. On allowing the jaws to spring open, they will engage the extractor and permit its handling.

The apparatus is set up as shown in Fig. 3. The

¹ SCIENCE, 86: 201, 1937.



Figs. 1-3

source of heat is a micro hot plate consisting of a cigar lighter element in series with a rheostat. The extraction is allowed to continue for half an hour or more. The extractor is removed, the level of the chloroform is adjusted to the 5 cc mark, and acetic anhydride is added to the 7 cc graduation. 0.1 cc of sulfuric acid are added and the color developed is compared in the usual way.

I wish to express my appreciation to Eimer and Amend, who constructed the apparatus and furnished the drawings.

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AN IMPROVED PROTOCOL FOR DILUTION SERIES

For some years, the author has been using a dilution protocol that avoids the inconveniences of the more commonly used dilution series, and has the following desirable properties:

- (1) A suitable number of steps (eight) in each decade of the dilution range.
- (2) Ease of preparation.
- (3) Accuracy in preparation.
- (4) The error, that is introduced by the fact that the liminal value of the phenomenon studied lies between the last negative tube and the first positive tube, is constant throughout the dilution range.
- (5) Simple numerical expression of the concentration.

The series is based on a geometrical progression of the reciprocals of the powers of the eighth root of ten, yielding eight equal logarithmic steps in the decade. It is prepared by mixing 2.9983 parts of the previous dilution with 1 part of water. The cumulative error, introduced by mixing 3 parts of the previous dilution with 1 part of water, is less than 0.1 per cent. in the decade, or much smaller than the presumptive error involved in the use of pipettes for making the dilutions. To avoid accumulation of error, it is desirable to make a preliminary series of dilutions of 1/10, 1/100, 1/1000 . . . for the start of each decade. Concentrations can be conveniently expressed in a logarithmic notation ($pD = -\log$ of the dilution) similar to the commonly used pH. A typical protocol is given in Table I.

Assuming that the actual liminal value of the phe-

TABLE I
PROTOCOL FOR A DILUTION SERIES BASED ON THE RECIPROCAL
OF THE POWERS OF THE EIGHTH ROOT OF TEN

Dilution	Concentration grams per 100 ml.	pD	Preparation of series	
			ml. of previous dilution	ml. of water
1/10.....	10.000	1.000	stock solution	
1/13.34....	7.499	1.125	3	1
1/17.78....	5.623	1.250	3	1
1/23.71....	4.217	1.375	3	1
1/31.62....	3.162	1.500	3	1
1/42.17....	2.371	1.625	3	1
1/56.23....	1.778	1.750	3	1
1/74.99....	1.334	1.875	3	1
1/100.....	1.000	2.000	1 of 1/10 stock solution	9
1/133.4....	0.7499	2.125	3	1
1/177.8....	0.5623	2.250	3	1
and continuing				

nomenon studied lies midway between the last negative tube and the first positive tube, the error of the result is constant over the entire dilution range at 14.3 per cent. of this mid-value. In the case of the commonly used dilution series, 1/10, 1/20, 1/30 . . . , this error varies from 5.26 per cent. to 33.33 per cent. and averages 12.59 per cent. Should it seem desirable to have the value of pD refer to this probable liminal mid-value, the series can be started with a stock solution that is 1.143/10 instead of 1/10.

Similar protocols could be developed based on the geometrical progression of the reciprocals of the powers of the other roots of ten. These protocols would be useful for the production of coarser or finer series of dilutions. The author has investigated those series from the square root of ten to the tenth root of ten, and none of them seem as convenient for application as the one described.

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BOOKS RECEIVED

- AUDUBON, JOHN J. *The Birds of America*. Pp. x + xxvi. 500 colored plates. Macmillan. \$12.50.
- CATTELL, R. B., J. COHEN and R. M. W. TRAVERS, Editors. *Human Affairs*. Pp. xi + 360. 17 plates. Macmillan. \$4.25.
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- WILLIAMS, SAMUEL R. *Experimental Physics*. Pp. 158. Illustrated. Ginn. \$1.00.