CLXX. A RAPID AND RELIABLE TEST FOR VITAMIN D.

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(*Received October 25th, 1926.*)

Zucker and Matzner [1923] have stated that rats kept on a typical high-calcium low-phosphorus rachitogenic diet develop marked faecal alkalinity, and that the administration of an antirachitic to rats in this condition causes the faeces again to become acid ($p_H < 7{\cdot}0$).

Although not specifically put forward as a means for determining antirachitic activity, its use for this purpose was suggested to us by the above authors, and after three years' experience we have found it to be of value when a more rapid test than those usually employed is required for routine purposes.

We have made use of the following technique.

Inbred albino rats from 20 to 40 days old and from 40 to 60 g. bodyweight are fed *ad lib.* on diet 401 [Zucker, 1924] which has the following composition:

Patent flour	85·0 %
Powdered egg albumin	10·0 %
Calcium lactate	2·8 %
Ferric citrate	0·2 %
Sodium chloride	2·0 %

with 2–3 g. fresh spinach leaves per animal per day.

It will be seen that this diet includes the addition of a reasonable amount of fat-soluble A. It has been shown by Chick and Roscoe [1926] that 2 g. of spinach, particularly in the winter, contain negligible amounts of vitamin D. We first used diet 84 [Sherman and Pappenheimer, 1921], but comparative experiments showed that the effects of the two diets were practically identical, while the animals on 401 seemed somewhat healthier and livelier than those on 84.

The cages used have a false bottom, consisting of a zinc screen with a mesh large enough readily to permit food to fall through, but small enough to retain the faeces. The faeces are collected before mid-day, any contaminated with urine being rejected; they are weighed, and placed in small stoppered cylinders with sufficient water to give a 2 % suspension. The faeces are well disintegrated with a glass rod, and vigorously shaken, and the suspension is then run through a loose plug of cotton wool, and poured into a hydrogen

electrode of the rocking pattern. The hydrogen ion concentration of the suspension is then determined electrometrically in the usual manner.

Determinations of this kind are made at three to four day intervals until the p_H of the faecal suspension is nearly or definitely alkaline (10 to 12 days). Determinations are then made every day, or every other day at least, until two consecutive readings give a mean determination of 7·3 or more, neither of the individual determinations being less than 7·2. The animal is now ready to be fed with the substance to be tested.

After the third day of administering the substance to be tested, readings are taken every day, until two consecutive readings give a mean value of p_H 6·7 or less, and neither more than p_H 6·8. For our own laboratory convenience we have considered the quantity of antirachitic substance necessary for this purpose to be an antirachitic unit.

1. *The rise in faecal p_H on the basal diet.* Since the test was first adopted by us, over 280 groups of 4 animals have been used. In no single case has the faecal p_H failed to reach a value well above 7·0. The time taken has been from 10 to 15 days, and the alkalinity has been maintained either till the end of the period of feeding the rachitogenic diet or until the animals have been submitted to antirachitic conditions.

2. *Maintenance of the alkaline p_H with supplementary doses of non-antirachitic substances.* The diagrams in Fig. 1 show the negative effects of administering glycerol, liquid paraffin, olive oil solutions of crude cholesterol from cod-liver oil, and an olive oil solution of recrystallised cholesterol (M.P. 148°).

3. *The restoration of acidic p_H by means of sources of antirachitic vitamin.* Fig. 2 shows the effect of administering cod-liver oil and irradiated cholesterol. The sample of oil in question had been found on four pairs of animals (2 males and 2 females) to give an average increase in calcification of 76 % over the controls, and to raise the average value of A/R [Chick and Roscoe, 1926] from 0·607 to 1·064.

4. *The restoration of an acidic p_H by means of irradiation.* Two pairs of animals, treated respectively for 15 and 30 minutes daily with the ultra-violet radiation from an open tungsten arc at 2 ft. distance, showed the change in faecal p_H illustrated in Fig. 3, curves 13 and 14.

5. *The prevention of the rise in faecal p_H by means of irradiation.* Two groups of two animals treated respectively for 15 and 30 minutes daily with the ultra-violet radiation from the tungsten arc at 2 ft. maintained their faecal acidity for 25 days, as shown in Fig. 3, curves 15 and 16.

We are aware that similar effects to those described above as a result of the administration of antirachitic substances have been obtained by the use of physiologically acidic substances, *e.g.* ammonium chloride [Zucker, Johnson and Barnett, 1922], or by considerably diminishing the calcium-phosphorus ratio of the diet [Sherman and Pappenheimer, 1921]. This does not, however, detract from the usefulness of the test in the case of substances of known origin, such as the extract of cod-liver oil to which we have been applying it for over two years.

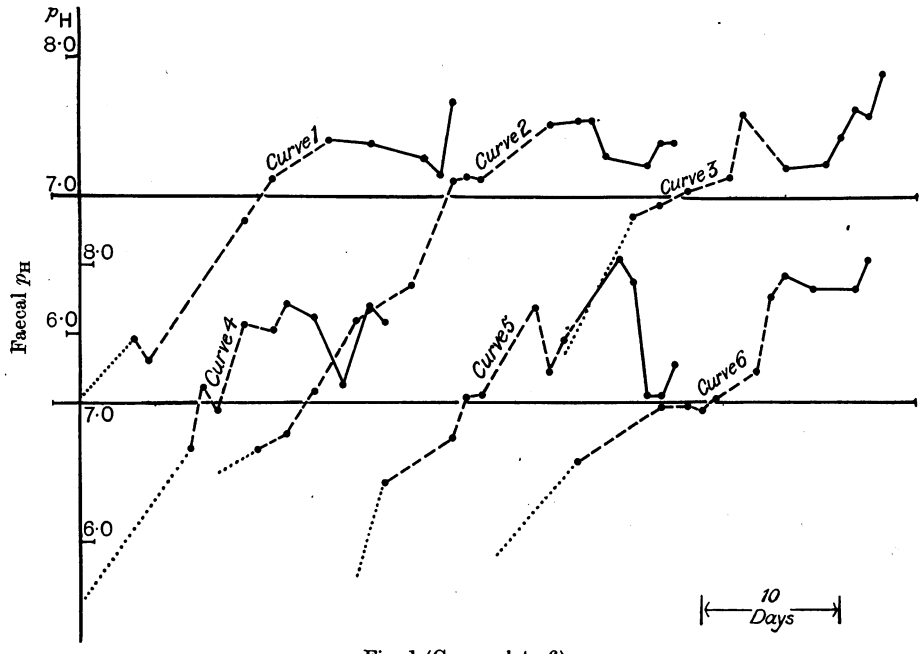

Fig. 1 (Curves 1 to 6).

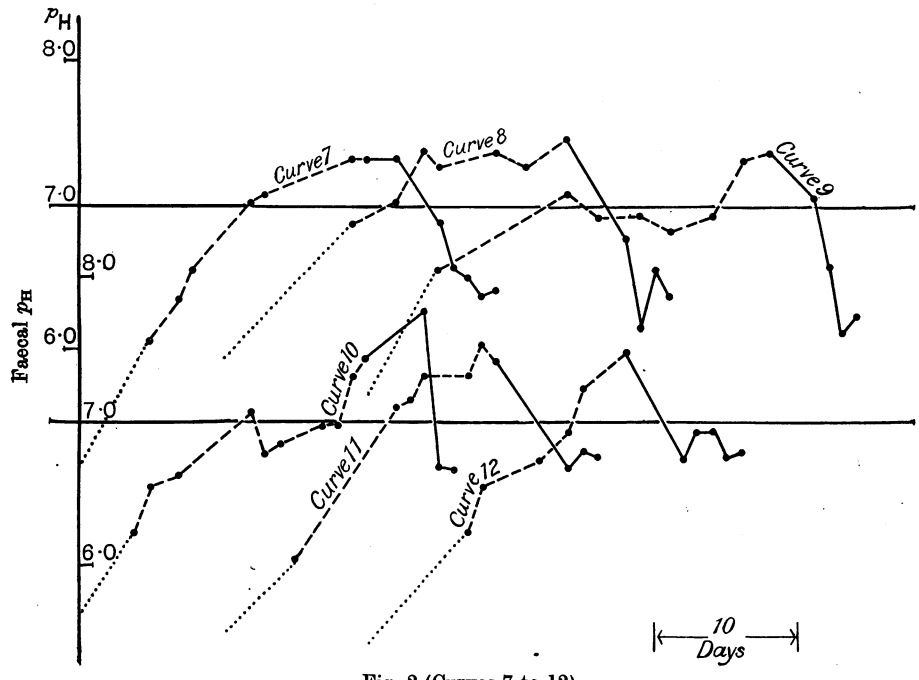

Fig. 2 (Curves 7 to 12).

– – – – – – – – = Period on basal diet alone.
——————— = Period on supplemented basal diet.
. . . . . = Period prior to first determination.

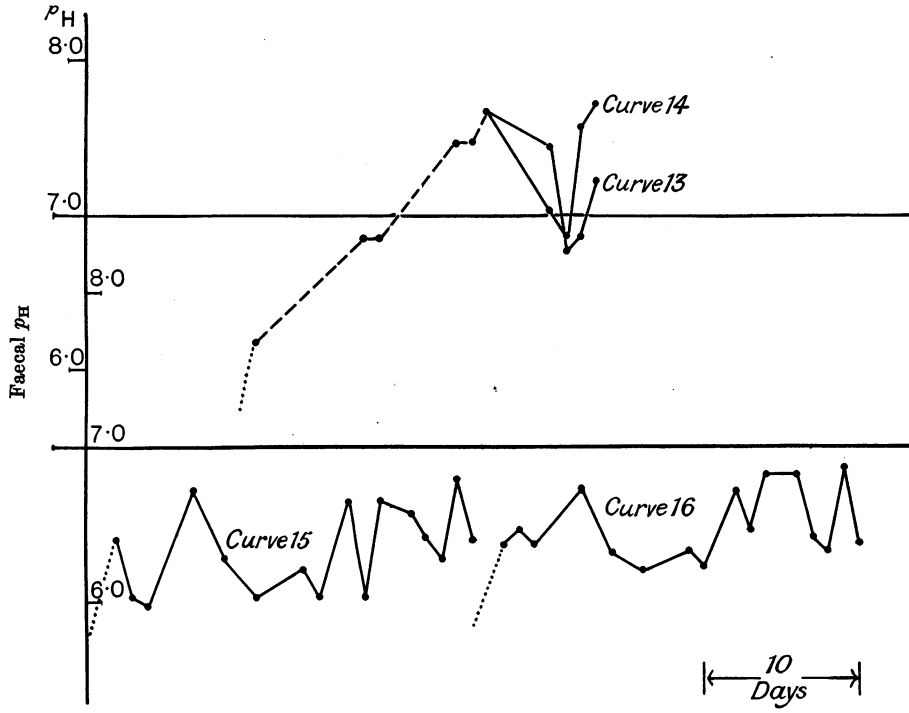

Fig. 3 (Curves 13 to 16).

---------- = Period on basal diet alone.

———————— = Period on supplemented basal diet.

..... = Period prior to first determination.

Daily supplementary treatment	Basal diet	No. of animals	Day of beginning supplementary treatment	Curve
Fig. 1				
0·04 cc. paraffinum liquidum, B.P.	401	2♀	18	1
0·025 cc. glycerol	84	2♂, 1♀	24	2
0·025 cc. glycerol	84	2♂	16	3
>0·001 g. recryst. cholesterol, in 0·02 cc. olive oil ...	401	2♀	15	4
>0·001 g. crude cholesterol, from cod-liver oil, in 0·02 cc. olive oil	401	2♀	15	5
>0·0015 g. crude cholesterol from cod-liver oil, in 0·02 cc. olive oil	401	2♀	22	6
Fig. 2.				
0·10 cc. cod-liver oil	401	1♂, 1♀	20	7
0·002 g. cholesterol, irradiated 1 hour, in 0·08 cc.	401	2♂	24	8
0·001 g. cholesterol, irradiated 2 hours, in 0·04 cc.	401	2♂	28	9
0·0005 g. cholesterol, irradiated 3 hours, in 0·02 cc.	401	2♀	20	10
0·00013 g. cholesterol, irradiated 4½ hours, in 0·08 cc.	401	2♂	19	11
0·002 g. cholesterol, irradiated 12 hours, in 0·08 cc.	401	2♀	18	12

All cholesterol was irradiated at 7½ inches from an open tungsten arc, and then dissolved in olive oil.

Fig. 3.				
Animals irradiated 30 minutes at 2 feet	401	2♀	16	13
Animals irradiated 15 minutes at 2 feet	401	2♀	16	14
Animals irradiated 30 minutes at 2 feet	401	1♂, 1♀	0	15
Animals irradiated 15 minutes at 2 feet	401	1♂, 1♀	0	16

The same arc was used for irradiating the animals as for irradiating the cholesterol. In the case of animals illustrated by curves 15 and 16, irradiation was omitted on the 6th and 13th days (Sundays).

SUMMARY.

1. Albino rats on certain high calcium-low phosphorus diets develop marked faecal alkalinity in 10 to 15 days.

2. This alkalinity is not affected by the administration of certain non-antirachitic substances.

3. The faecal p_H can be reduced to the acid side of neutrality by means of cod-liver oil, irradiated cholesterol or irradiation.

4. The rise in faecal p_H on this diet can be prevented by irradiation.

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