PYRUVIC ACID (MGM. PER 100 GM. OF BLOOD)

Controls					Avitaminosis				
Time of introduction of the oil (1 ml.)									
Basal amounts	1 hr.	2 hr.	4 hr.	6 hr.	Basal amounts	1 hr.	2 hr.	4 hr.	6 hr.
0·94 1·07 1·65 0·64 0·97 1·07 1·22 0·93 0·99 0·70	0·97	1·18	1·11	1·20 0·68 0·75 0·79 0·98	2·70 2·29 1·83 4·05 1·29 1·42 1·89 1·80 2·27 1·97 1·28 1·51 2·86	2·40 1·15 1·27	1·93 1·35 2·01	1·14 1·28 2·75	0·94 0·89 1·07 1·31 1·19 1·09 0·72 0·44 0·47
Av. 1·01 ± 0·28				0·89 ± 0·21	2·10 ± 0·77				0·90 ± 0·3

of aneurin in the hearts of rats getting more than 2 μgm. of aneurin a day increases with the fat content of the diet; but they also point out that the increase following the addition of 10 per cent of fats is larger than that obtained by administering 20 per cent of fats. They believe that "the data do not necessarily indicate that dietary fat exerts a sparing action on thiamine requirements". Gruber[3] states precisely the meaning to be given to the expression 'sparing action of fat vitamin B_1', indicating that it is to be interpreted as due to a decreased call for vitamin in metabolic processes. In fact, some of Gruber's researches (pigeons) show that the increased metabolism of carbohydrates induced through a larger introduction of these substances causes a quicker disappearance of aneurin pyrophosphate from the tissues.

We have studied the effects of fats from another point of view, namely, that of the pyruvic acid content of the blood. The work is based on the fact that, through the fundamental researches by Peters[4,5] and his school, as well as those on man and animals by Lu[6], it can be considered as proved that close relations exist between avitaminosis B_1 and the accumulation of pyruvic acid both in poultices of tissues *in vitro* and in tissues and blood *in vivo*. Our present investigations are part of a larger group of researches, the aim of which is to study what influence the administration of carbohydrates (glucose), amino-acids and fats can have on the pyruvic acid of the blood of animals suffering from avitaminosis B_1.

Rats of the initial weight of 55–60 gm., kept on a synthetic diet deprived of vitamin B_1 (wheat starch 65 per cent, caseine deprived of fat and washed 18 per cent, olive oil 10 per cent, cod liver oil 2 per cent, Osborne and Mendel's saline mixture 5 per cent), with appropriate quantities of other vitamins belonging to group B (riboflavine, pyridoxine, niacine, pantothenic acid, biotin, inositol and choline), were used when the weight of the rats, after increasing during two to three weeks, came down again to a little more than the initial rate. Besides being controlled by their weight, the state of avitaminosis of these rats was checked by the amount of pyruvic acid in their blood.

The pyruvic acid was estimated by Lu's method as modified by Fornaroli[8]; blood was collected within 30 sec., and the blood withdrawn, collected on a little sodium fluoride, was immediately treated with freshly prepared 12 per cent trichloracetic acid.

In order to study the influence of fats on the pyruvic acid of the blood, 1 ml. of olive oil was introduced, by means of a sound, into the stomach of the animals suffering from avitaminosis which had been kept fasting for 12 hr. These animals were then sacrificed by decapitation, generally after 6 hr. In a limited number of cases they were sacrificed after 1, 2 or 4 hr.

The accompanying table shows the amount of pyruvic acid found in the blood of a series of rats.

The results clearly show that after 6 hr. from the introduction of the oil, the pyruvic acid in the blood of animals suffering from avitaminosis B_1 falls again to its normal amount. This decrease begins at the sixth hour from the introduction of the oil, showing that the effect takes place after absorption of the fat; in fact, after 6 hr., no significant quantity of oil was found in the stomach of the animals. A very small decrease, of which we are not yet sure and which requires further investigation, has also been noticed in normal rats 6 hr. after the administration of oil.

The exact return to the normal amounts of pyruvic acid in the blood of rats suffering from avitaminosis, after the administration of fat, seems clear evidence of the sparing action caused by fat. The organism finds itself with excess lipids and consequently metabolizes fats in preference to carbohydrates and proteins. As we have already shown, glucose and amino-acids administered to animals suffering from avitaminosis B_1 cause a noticeable increase of pyruvic acid in the blood.

The quick fall of pyruvic acid in the blood is in accordance with the interpretation of the sparing action of fats, showing that aneurin does not share in the fermentative systems involved in the metabolism of fats[9].

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Dihydroxyanthranilic Acid as a Precursor of Nicotinic Acid

A MIXTURE of 400 mgm. of *o*-aminoveratric acid (prepared by the method of Pschorr and Sumuleanu[1]), 100 mgm. of red phosphorus and 8 ml. of hydriodic acid (sp. gr. 1·7) was heated in a sealed tube at 100° for eight hours and then at 105° for two hours. The hydriodic acid salt of 2-amino-3,4-dihydroxybenzoic acid crystallized on cooling. After filtration, this product was recrystallized from a small amount of hydriodic acid containing water and obtained as macroscopic prismatic needles (m.p. 220°; found: N, 4·29; $C_7H_8O_4NI$ requires N, 4·71 per cent). The hydriodide of dioxyanthranilic acid was easily

dissociated by washing with a larger amount of water, and the free acid was recrystallized from alcohol (m.p. 174°; found: N, 8·05; $C_7H_7O_4N$ requires N, 8·29 per cent).

8 gm. of liver slices of cattle, pigs and horses prepared immediately after killing was incubated with the mixture of 5 mgm. of L-tryptophane or of another substance (in the case of other substrates an equimolecular amount to tryptophane was weighed) in 3 ml. of physiological saline solution and 5 ml. of *M*/15 phosphate solution (*p*H 7·5) for 24 hr. Its nicotinic acid content was determined by the method of Swaminathan[2] and Kawashima[3] with bromcyan and aniline.

This chemical method is based upon the colour reaction of the anil which is formed by interaction between aniline and a carbonyl compound, a fission product of the pyridine ring with bromcyan. This colour reaction is so specific that in the case of α-substituted pyridine compounds, for example, quinolinic acid and pyridoxin, this colour reaction becomes negative. Accordingly, if a substance derived from tryptophane gives this colour reaction, the compound in question may be said to be nicotinic acid.

Table 1

*p*H	Increase of nicotinic acid (mgm.)			
	Horse liver		Cattle liver	
	Quinolinic acid	Tryptophane	Quinolinic acid	Tryptophane
6·0	0·24	0	0·13	0·09
7·5	0·35	0·14	0·53	0·56
8·1	0·24	0·12	0·17	0·13

Under our experimental conditions, the yield of nicotinic acid from tryptophane was greatest at *p*H 7·5, and to both sides from this *p*H the production of nicotinic acid rapidly decreased. The yield of nicotinic acid increased proportionately to the amount of substrate up to 2 mgm. of tryptophane; above this amount the increase of nicotinic acid is very small.

Table 2. Experiment with Cattle Liver

Substrate	Amount of substrate added (mgm.)	Increase of nicotinic acid (mgm.)
Tryptophane	5	0·30
Anthranilic acid	3·4	0
3-Hydroxyanthranilic acid	3·9	0·73
3,4-Dihydroxyanthranilic acid	4·2	0·73
Quinolinic acid	4·1	0·38
Control	0	0

All the results recorded in Tables 1, 2 and 3 were obtained in the hot summer season (July and August, 1950). In winter the yield of nicotinic acid produced by the liver enzyme from tryptophane seemed to be twice or three times as much.

Table 3. Experiment with Horse Liver

Substrate	Amount of substrate added (mgm.)	Increase of nicotinic acid (mgm.)
Tryptophane	5	0·14
Anthranilic acid	3·4	0
3-Hydroxyanthranilic acid	3·9	1·24
3,4-Dihydroxyanthranilic acid	4·2	1·04
Quinolinic acid	4·1	0·35
Control	0	0

Anthranilic acid, which was added to the cattle liver slices, did not produce nicotinic acid. 3-Hydroxyanthranilic acid and 3,4-dihydroxyanthranilic acid were also used. Both acids have almost the same ability to produce nicotinic acid, and the yield exceeded considerably that from tryptophane.

According to Henderson[4], rats excrete quinolinic acid in the urine after administration of a larger dose of tryptophane. In our own clinical investigations the quinolinic acid cured pellagra, though a much larger dose of it was necessary than of nicotinic acid.

However, the amount of nicotinic acid produced from quinolinic acid is less than from 3-hydroxyanthranilic acid and almost the same as that of tryptophane; and it is obvious that 3,4-dihydroxyanthranilic acid must be taken into consideration as one of the precursors of nicotinic acid.

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Distribution of Vitamin C in the Tip of the Broad Bean Radicle

It was stated in an earlier communication[1] that if 4-mm. portions of the broad bean radicle were assayed for vitamin C, the ninth portion from the tip contained an average of 112·96 mgm. of the vitamin per 100 gm. of fresh weight. This amount was thought to be present within the region of maximum growth. Following this statement, J. Read pointed out to us in a private communication that the accepted region of maximum growth occurs at a distance of 5–7 mm. from the tip. He suggested that analysis of one-millimetre portions should therefore be undertaken.

The rise in the vitamin C content of the radicle shown in the previous communication[1] is within an actively growing region, since it is here that the secondary roots are being formed; but the expression "region of maximum growth" does appear to be misleading.

One-millimetre portions of radicle have now been assayed, and the results for the first ten millimetres are given in the accompanying graph. The beans were germinated in damp sand and the radicles sus-