IV. FLAVIN CONTENT OF ANIMAL TISSUES UNDER DIFFERENT CONDITIONS.

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A NUMBER of communications during recent years by Warburg and Christian [1932, 1, 2] show that besides the iron-containing enzyme of a haemin nature there exists another enzyme capable of transporting oxygen under aerobic conditions and of dehydrogenation under anaerobic conditions. The scheme of its action is as follows:

1. Leuco-enzyme + oxygen = yellow enzyme + hydrogen peroxide (under aerobic conditions).
2. Leuco-enzyme + methylene blue = yellow enzyme + leucomethylene blue (under anaerobic conditions).

The power of flavins to oxidise by means of dehydrogenation has also been confirmed by Wagner-Jauregg *et al.* [1934] and by Adler and Euler [1934]. Freed from its colloidal carrier the yellow oxidation enzyme is identical with the flavin discovered and described in the same year by Kuhn *et al.* [1933, 1, 2] and simultaneously by Ellinger and Koschra [1933]. These same authors have shown in their investigations that the flavins are quite similar to vitamin B₂ in their action on the organism, and this makes these compounds the more important. Notwithstanding the great biological significance of the flavins, there do not exist sufficiently precise methods for their determination in animal tissues and organs. György *et al.* [1934] have drawn up a table of the flavin content of tissues according to their degree of action as carriers of vitamin B₂. Euler and Adler [1934] have compared the intensities of the colour of aqueous extracts from different tissues with a standard scale of solutions of flavins in different concentrations. The data obtained by Euler agree well with the data obtained by György *et al.* These methods however do not supply absolute values of the flavin content, but give only approximations.

We believe the method of Warburg and Christian [1933] to give more exact determinations. These authors consider it possible to calculate the concentration of flavins by the intensity of the absorption spectra of methanol extracts. This method of determination is however hardly available for most laboratories as it requires complicated equipment; we have therefore modified it by substituting colorimetry for spectrophotometry.

Method of determination.

Our method of determination is as follows. A definite amount of the organ is powdered and repeatedly washed in water until the water remains clear. The tissue is then extracted with 10 parts of a mixture of 3 vols. methanol with 1 vol. water for 36–48 hours at 28–36°. The extract is centrifuged and the upper transparent layer, containing all the flavins of the tissue under examination, is decanted into a separating funnel and treated with an equal volume of

chloroform. The other pigments associated with the flavins pass into the chloroform; treatment with chloroform also clarifies the methanol layer. The lower chloroform layer, usually uncoloured (if the flavins of the kidneys, heart and liver are being examined), is drawn off, and the methanol layer is used for the determinations. The yellow flavin solution in methanol, having a green fluorescence, is compared in a colorimeter with a standard solution of potassium chromate containing 0.1 mg. KCrO_4 in 1 ml. This solution corresponds in colour to 1 ml. of an extract containing 2.37% of flavin as shown below. Colorimetric determinations are possible for two reasons: (1) methanol extracts of the liver, kidneys, heart, brain or lungs do not contain any other pigments, as has been demonstrated by the absorption spectra of extracts of these organs; (2) at these concentrations the solution behaves according to Beer's law that the intensity of the colour is proportional to the concentration of the substance. In Table I is given an example of a determination of the flavin in the cortical substance of the kidney. 9.4 g. of the tissues were treated as above described. The volume of the methanol extract after treatment with chloroform was 70 ml. The solution was yellow-green in colour. The absorption spectrum was determined in the usual manner according to Warburg [1933].

SPECTROPHOTOMETRIC DATA.

Table I. *Measurements of the absorption of light by a methanol extract of the cortical substance of the kidneys.*

λ $m\mu$	I_0	I_1	$\frac{I_0}{I_1}$	λ $m\mu$	I_0	I_1	$\frac{I_0}{I_1}$
510	27.5	25.5	1.08	450	3.6	1.95	1.85
500	21.5	18.2	1.18	448	2.95	1.55	1.91
490	15.7	11.9	1.32	445	2.8	1.5	1.86
430	12.4	8.3	1.47	440	2.1	1.2	1.75
470	8.5	5.2	1.64	430	1.127	0.7	1.61
460	5.9	3.3	1.78	420	0.7	0.5	1.5
455	3.5	1.85	1.88	415	0.35	0.25	1.4

As the table shows, maximum absorption occurs at a wave-length of 448 $m\mu$ in the visible spectrum. This fully agrees with the data obtained by Warburg and Christian [1933] for lactic acid bacilli and yeast. The flavin concentration calculated by the formula of the above authors was 0.2406 mg. in the 70 ml. extract or 25.6% per g. of kidney cortex.

The methanol extract was then measured by the colorimetric method. The standard solution, as has been mentioned above, contains 0.1 mg. KCrO_4 in 1 ml. Comparison showed that the intensity of the colour of 1 ml. of the extract corresponded to the colour of 0.145 mg. KCrO_4 in 1 ml. But 1 ml. of extract contains 0.2406/70 mg. or 3.44% of flavin. Hence 0.1 mg. KCrO_4 in 1 ml. will correspond in colour to 3.44/1.45 or 2.37% flavin in 1 ml. This method is suitable for making determinations of flavins in those tissues and organs where the aqueous methanol extract does not contain other pigments, colouring the solution yellow. The liver, kidney (cortical substance), heart and brain belong to such tissues. Other tissues such as the spleen and, especially, blood contain a number of other pigments which influence the colorimetric determinations. Spectrophotometric analysis of the extract of the blood of the white rat for example clearly demonstrates the presence of three maxima, probably corresponding to the haemins (419 $m\mu$, 474 $m\mu$ and 575 $m\mu$). It is evident therefore that colorimetric determination of flavins in blood cannot be based on the yellow colour of aqueous methanol extracts.

Flavin contents of various organs.

Using this method, so convenient for its rapidity and precision, we determined to have the flavin contents of the organs and tissues of several animal species. In Table II the flavin contents of the organs and tissues of a cat and a rabbit are given.

Table II. *The flavin contents in γ per g. of tissues and organs of a rabbit and a cat.*

Species	Liver	Kidney	Kidney cortex	Kidney medulla	Heart	Lung	Brain	Muscle
Rabbit	7.17	14.5	—	—	6.15	9.90	3.07	—
Cat	24.5	6.56	25.2	0	7.82	—	—	—

Our data on the distribution of flavins in the tissues and organs agree with the data of Euler and Adler [1934].

Effect of avitaminoses-A and -C.

We then carried out a series of experiments to determine the effect of avitaminosis on the flavin content of the animal body. Abderhalden's earlier work [1921] on the influence of avitaminosis in lowering the respiration of tissues served as the starting point for our experiments. The flavins form an oxidation-reduction system existing in every cell. We therefore considered it interesting to establish how much avitaminoses-A and -C influence the flavin contents of the livers of calves, rats and guinea-pigs. Determinations of the flavin contents of the liver and kidneys only were made, since these organs are richest in flavins. Table III summarises the results of our experiments.

Table III. *The flavin content in γ per g. of liver and kidneys of animals suffering from avitaminoses.*

Species	Normal	Avitaminosis-A	Avitaminosis-C
Calf (liver)	35.6	28.4	—
Rat (liver)	—	16.9	—
„	—	28.4	—
„	30.0	27.5	—
Guinea-pig			
Liver	20.8	—	22.0
„	13.7	—	19.9
„	11.9	—	—
Kidney	15.6	—	14.9
„	—	—	19.2

A small decrease in the flavin content appears to occur in the liver of animals suffering from avitaminosis-A; avitaminosis-C however does not give a clear result; an increase in the flavin content occurred in the liver and a decrease in the kidneys.

Effect of rich protein diet.

The work of Tcherkess [1934] indicates that the lesions of pellagra are often caused by a faulty balance of proteins in the diet and sometimes by excessive protein in the diet of experimental animals. A high content of protein in the food exercises also a large specific dynamic effect. Wishing to establish to what extent these phenomena react on the course of oxidation-reduction processes, we made a number of experiments to determine the influence of a rich protein diet

on the flavin content of the liver of white rats. The rats were sub-divided into two groups of eight animals in each, a control and an experimental group. The control group received the usual laboratory diet while the experimental group was fed on a diet rich in protein. The diet of this experimental group consisted of:

60 parts caseinogen (treated with alcohol);
 40 parts starch (rice);
 10 parts dry yeast;
 1 part irradiated yeast;
 4.8 parts Mendel-Osborne salt mixture.

The rats were kept for 28 days on such a diet. Table IV gives the flavin content in the liver of the experimental and control rats.

Table IV. *The effect of a rich protein diet on the flavin content
 γ per g. of liver of white rats.*

No.	1	2	3	4	5	6	7	8
Control	—	19.3	18.2	20.7	13.2	21.0	21.5	19.6
Experimental	20.3	20.4	19.2	18.5	22.9	21.6	19.3	20.3

The quantity of flavins in the liver does not apparently change significantly under the influence of a rich protein diet.

SUMMARY.

1. A method of determining flavins in some tissues and organs is described.
2. Data of the flavin contents of the tissues and organs of a cat and a rabbit are given.
3. In avitaminosis-A there is observed a slight decrease in the flavin content of the livers of white rats and calves.
4. The influence of avitaminosis-C is not clear.
5. A rich protein diet also has no definite effect on the flavin content.

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