Table I.–Counts of grains per 64 μ^2 in the retina.

1 Outer layer of rods and pigment epithelium	2 Inner layer of rods	3 Outer nuclear layer	4 Outer plexiform layer	5 Inner nuclear layer	6 Inner plexiform layer to the layer of optic fibres
68·24 ± 2·04	40·36 ± 1·04	20·92 ± 1·01	32·78 ± 1·32	25·48 ± 1·31	38·58 ± 1·35

grade cross-reactions with mesodermal tissues is probably caused by the non-removal of some ciliary muscles during the preparation of the antisera.

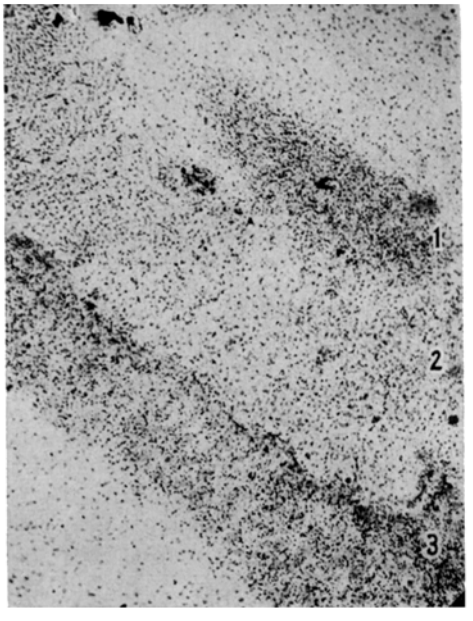

Fig. 5.–Autoradiograph of part of retina and external tissues (unstained) (× 260). *1* = optic nerve; *2* = extrinsic muscles of the eye, chlorid, sclera; *3* = retina. The width of the retina here is about ⅓ of that of the region in Figures 2 and 3.

As might be expected, the activity of the lens was in all cases higher than that of the retinal layers.

Table II.–Counts of grains per 64 μ^2 in the cornea.

Epithelial layer	Fibrous cornea
23·53 ± 1·07	11·63 ± 0·91

Table III.–Counts of grains per 64 μ^2 in the muscles of the eye.

Ciliary muscle	Extrinsic muscle of the eye
26·86 ± 1·06	31·00 ± 1·02

The formation and distribution of these antigens during ontogeny is now being studied. Investigations are also in progress on the changes in quantity of lens specific protein (tested by anti-lens sera after absorption) during induction, development and regeneration.

Acknowledgements. One of us (M.F.) gratefully acknowledges the award of a British Council scholarship. We are indebted to Professor C. H. Waddington for his interest and encouragement, and to several of our colleagues and the technical staff of the Institute of Animal Genetics for assistance with the freeze-drying apparatus, section-cutting, photography, etc.

R. M. Clayton and M. Feldman

Institute of Animal Genetics, Edinburgh, and Department of Zoology, Hebrew University, Jerusalem, July 18, 1954.

Résumé

Des anticorps contre le cristallin et contre le muscle cardiaque de la souris ont été marqués par le radio-iode, puis employés pour la localisation histochimique et pour une analyse quantitative de ces antigènes au cours du développement.

Studies on Factors Influencing the Endogenous Respiration of Liver Homogenates

The Action of Nicotinamide on a Keto-oxidasic Activity of Rat's Liver

We have previously shown how nicotinamide added to a homogenate of rat's liver is capable of increasing endogenous respiration, under particular experimental conditions[1].

As Feigelson and others[2] had previously demonstrated an inhibiting, and not excitatory, action of nicotinamide on endogenous respiration, it was necessary to establish the reason for this divergence in data. We wished to find out if the diet had any influence on these phenomena in order to solve this problem. We have therefore studied the influence of nicotinamide on the endogenous respiration of liver homogenates obtained from (a) rats on a normal diet balanced according to Randoin and Couseret's dictum[3], and the same in a fasting condition, (b) animals fed on a hypoprotein and hyperlipidic diet. The technique used was that already described in previous works[1].

The results of these experiments is that the hypoproteic (hypoprotidic hyperglycidic and hypoprotidic-hyperlipidic) diet and the fasting state (in animals fed on a balanced diet) are the cause of conditions which show up the phenomena already described by us, i.e. endogenous respiration values in the presence of nicotinamide become much higher than those from homogenates without nicotinamide (Fig. 1 and 2). The hyperlipidic diet is responsible for the maximum intensity of the phenomenon (Fig. 1).

[1] L. Villa and N. Dioguardi, Medicina *3*, 287 (1952); Boll. Soc. Lomb. Sci. Med. Biol. *7*, 405 (1952); Com. Congr. internaz. Chim. Biol., Parigi, luglio (1951); Exper. *9*, 469 (1953).

[2] F. Feigelson, J. N. Williams, Jr., and C. A. Elvehjem, J. Biol. Chem. *189*, 361 (1951).

[3] L. Randoni and G. Couseret, Bull. Soc. Sci. d'Hyg. Alim. *1*, 14 (1947).

Hyperprotidic and balanced diets completely abolish the excitation of endogenous respiration due to nicotinamide and, further more, the addition of this compound causes a reduction (Fig. 2).

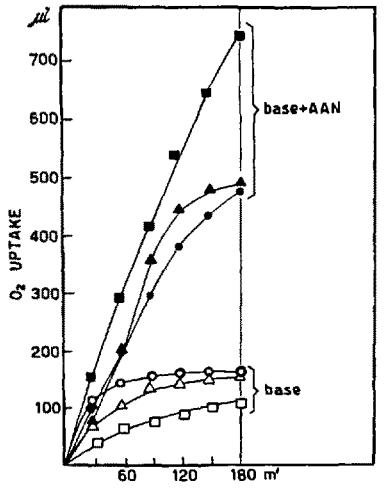

Fig. 1.—The endogenous respiration of homogenates obtained from the liver of rats on a hyperprotidic diet (□hypoprotidic-hyperlipide, Δ hypoprotidic-hyperglycidic), and rats fasting 48 h from a balanced diet (°). Homogenized in 5 parts of isotonic KCl and Ringer-phosphate solution (pH 7·0) to 3 cm³. Doses of 1×10^{-3} mol of nicotinamide (AAN in the tigine) were placed in the principal cavity. Central well — 0·2 ml. 2/*M* KOH, Atmosphere: air. Temperature of the bath, 30°C. The flasks were kept in finely crushed ice until they were put in the bath.

We have also studied whether the addition of malonate had an important inhibiting role on the phenomenon observed under these conditions. Experiments dealing with this problem have supplied us with data from which it is possible to assert that malonate does not inhibit nicotin amide excitation (Table II).

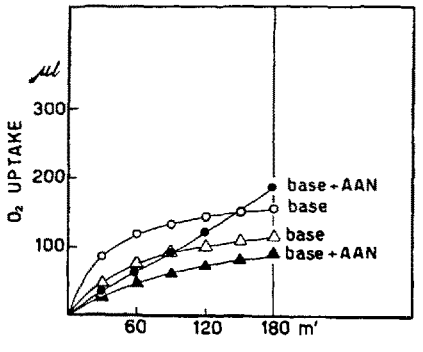

Fig. 2.—The endogenous respiration of homogenates obtained from the livers of rats on a balanced diet (°) and on a hypoprotidic diet (△). The experimental conditions are the same as for Figure 1.

We have also tested the influence of various oxidizable substances, with and without nicotinamide, on liver homogenates from rats fed on a balanced diet and not starved. This was carried out in order to try to identify the oxidizable substrate responsible for the increased O_2 consumption. The data are summarized in Table I. The study of the activity of liver homogenates from rats maintained on a balanced diet, without fasting, in the presence of numerous oxidizable substrates, allows us to affirm that only with aceto-acetate was it possible to repeat the phenomenon observed in the case of liver homogenates obtained from rats on hyperprotidic diets

Table I.—Experiments carried out in normal Warburg Flasks with a side arm: homogenate obtained with 5 parts isotonic KCl; nicotinamide 1×10^{-3} mol in the main cavity; substrate in side arm added after 10 min equilibrium in thermostatic bath, in concentrations given at the foot of the table. Phosphate Ringer buffered at pH 7·0, up to 3 ml, 0·2 ml, 2 *M* KOH in central well. Temperature of bath 30°C; time of experiment: 3 h. The data reported are those obtained after 1 h. [1]

Substrate	Without nicotinamide		Without nicotinamide	
	XO_2 base	XO_2 substr.	XO_2 base	XO_2 substr.
Succinate (°)	76	490	54	265
Alpha-ketoglutarate (°)	50	78	37	53
Alpha-ketoglutarate (°) + malonate (°°)	–	69	–	32
Malate (°)	65	76	48	45
Fumarate (°)	58	66	32	36
Pyruvate (°)	71	77	39	45
Pyruvate + malate (°)	55	68	40	42
Lactate (°)	62	79	30	34
Citrate (°)	81	96	95	107
β-hydroxybutyrate (°)	60	96	51	56
β-hydroxybutyrate (°) + malate (°°)	71	86	58	77
Propionate (°)	67	74	44	44
Propionate (°) + malate (°)	85	99	63	87
Acetate (°)	71	89	48	47
Acetate (°) + malate (°)	66	30	55	30
Acetocetate (++)	120	150	200	430
Valine (+)	43	43	62	42
Serine (+)	58	48	68	46
Cystine (+)	64	32	48	30
Methionine (+)	44	35	59	30
Glycocoll (+)	59	53	78	53
Proline (+)	72	44	63	40
Oxyproline (+)	64	30	50	33
Glutamate (+)	48	35	53	32
Aspartate (+)	69	49	68	47
Glucose (_)	54	46	55	47
Xylose (_)	63	50	56	48
Ribose (_)	48	30	49	33

(°) = 1 mol × 10^{-3} (+) = 1 mol × 10^{-4}
(°°) = ⅓ mol × 10^{-3} (—) = 2 mol × 10^{-3}
(++) = data after 3 h of experiment

Table II.—The oxidation of aceto-acetate, in presence of nicotinamide, by liver homogenates obtained from non-fasting rats maintained on a balanced diet. The aceto-acetate, extemporaneously prepared by the method of LJUNGGREN[2], was placed in the side arm and added to the mixture ten minutes after equilibrium. The experimental conditions are the same as for Table I. Those are the 75% positive data. In 25% of our experiments we were not able to produce the aceto-acetate oxidation.

Base	130 ± 15
Base + nicotinamide	140 ± 20
Base + aceto-acetate	130 ± 10
Base + aceto-acetate + nicotinamide	420 ± 45

[1] P. I. MANN and J. H. QUASTEL, Biochem. J. *35*, 502 (1941).
[2] G. LJUNGGREN, Chem. Zbl. *2*, 23 (1924).

Table III.—Homogenate obtained with 5 parts of isotonic KCl; nicotinamide 1 × 10^{-3} mol malonate ⅓ mol × 10^{-3} in the main cavity. Phosphate Ringer buffered at pH 7·0 up to 3 ml 2 *M* KOH in the central well. Temperature of bath 30°; duration of experiment 3 h. Data of 3 h of experiment.

	Without nicotinamide	With nicotinamide
Base	160	390
Base ± malonate	148	380

or fasting from balanced diets. The oxidation of aceto-acetate in the presence of nicotinamide is carried out according to the properties shown in Table II.

It is possible to conclude from these data that:

(a) nicotinamide increases the endogenous respiration of a liver homogenate when the latter is in a state of increased lipidic catabolism.

(b) the substrate for this action seems to be aceto-acetate. It is possible to put forward the hypothesis, that this occurs through enzymes bound together by a metabolism of the ketonic oxidasic type, particularly that involving aceto-acetate. We have also noted that the phenomenon of nicotinamide excitation is not definitely influenced by the presence of malonate (Table III).

These data have caused us to think that the phenomenon occurring in presence of nicotinamide is, broadly speaking, bound up with a protective type of action towards a keto-oxidasic system, precisely aceto-aceto-oxidase, similar to that already known for DPN in regard to DPN-ase.

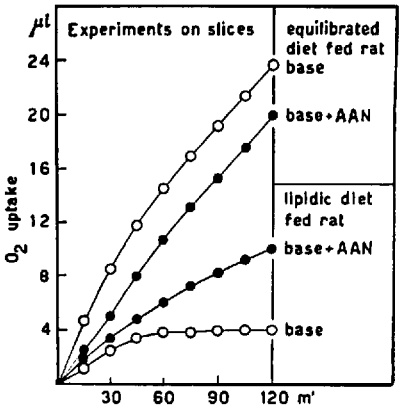

Fig. 3.—The endogenous respiration of liver slices obtained from rats maintained balance and on a steatogenous diet for 20 days. These slices were suspended in up to 3 ml Ringer-phosphate solution. 1/2 × 10^{-4} mol nicotinamide (A.A.N. in the figure) was placed in the principal cavity. Central well 0·2 ml. 2/*M* KOH. Atmosphere: air. Temperature of the bath, 30°C. The data are expressed for mg dry tissue.

In order to have an idea as to whether the phenomenon took place even when the tissues were under more physiological conditions, experiments, limited to the two pre-eminently interesting conditions: hepatic steatosis due to prolonged hypoprotidic-hyperlipidic alimentation and normal livers on a balanced diet, without fasting, were carried out using liver slices instead of homogenates.

Nicotinamide increased the basic consumption of oxygen for the liver in a state of steatosis while it did not stimulate, but rather inhibited, that of the normal liver (Fig. 3).

Summarizing our researches it is possible to conclude that probably the object of the intensified activity on the part of the liver slices and homogenates, in presence of nicotinamide, is aceto-acetate.

It is therefore necessary to postulate the existence of a keto-oxidasic system in rat's liver, and more precisely an aceto-aceto-oxidase system, the activity of which is only demonstrated under particular research conditions, i.e. when the enzymatic source is protected with nicotinamide.

L. Villa and N. Dioguardi

Institute of Clinical General Medecine and of Therapy, University of Milan, July 5, 1955.

Zusammenfassung

Es gelang den Autoren, in der Rattenleber ein Azeto-azeto-oxydase-System nachzuweisen, in welchem dem Nicotinamid eine Schutzwirkung zukommt.

The Influence of Progesterone on Adenosintriphosphatase

It was shown in a previous paper[1] that ATPase activity of myofibrils and mitochondria isolated from both guinea pig heart and muscle is inhibited by water suspensions of 1·4 × 10^{-4} progesterone and by aqueous solutions of desoxycorticosterone at the same concentration.

Jones and Wade[2] have, however, recently reported that ATPase activity of rat liver homogenates is strongly enhanced by progesterone. In these experiments the liver was stored at − 30° C before use.

Since the difference in the experimental conditions may have exerted an effect on the difference of results, the problem was studied again, by adopting the same technique as used by Jones and Wade. The results of this study are described in this note.

Albino rats weighing 150–170 g, fed with a standard diet including all vitamines and dietary factors, were used as experimental animals. They were killed by dislocation of the head, the livers and the hearts immediately taken out, weighed and transferred into the cold room at 2°C. 10% homogenates were prepared with 0·25 *M* sucrose or water as suspension media. Homogenization of the liver was made in a Potter-Elvelyem glass apparatus. Mitochondria were isolated by differential centrifugation in the Serval type SS–1 Angle Centrifuge (30 min at 1200 × g, after a first centrifugation at 1500 × g for 10 min which removed cellular débris, nuclei, damaged cells and red cells). Heart homogenates were prepared by cutting the entire organ with a freezing microtome, the width of each section being about 12 μ. The sections were put in a Waring blendor for 20 min and mitochondria were then isolated from this homogenate according to the procedure described in a previous paper[1].

ATPase activity was determined according to the method of Dubois and Potter[3], with 0·067 *M* borate buffer, pH 6·9. 0·1 ml of 10% homogenates and mitochondrial suspension were used as enzyme material. Concentration of ATP was 0·003 *M* and final volume of the reaction mixture 3 ml. Incubation temperature was 37°C and time of experiment 15 min. After this time the

[1] M. A. Mor, Exper. *9*, 342 (1953).
[2] H. W. Jones and R. Wade, Science *118*, 103 (1953).
[3] K. P. Dubois and V. R. Potter, J. Biol. Chem. *150*, 185 (1953).