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.

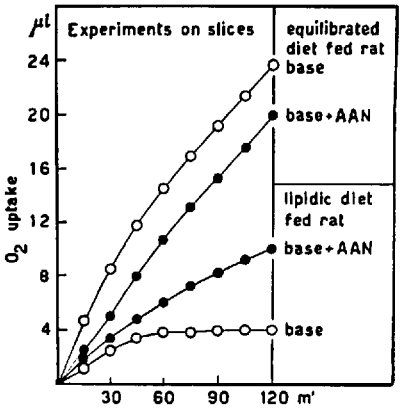

Fig. 3.—The endogenous respiration of liver slices obtained from rats maintained balance and on a steatogenous diet for 20 days. These slices were suspended in up to 3 ml Ringer-phosphate solution. 1/2 × 10^{-4} mol nicotinamide (A.A.N. in the figure) was placed in the principal cavity. Central well 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.

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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).

Table I.–ATPase activity of normal rats without storing of organs at 30°C
(γ P/mg N)

Liver homogenates in H_2O		Liver homogenates in 0·25 *M* sucrose		Liver mitochondria in 0·25 *M* sucrose		Heart mitochondria in buffer borate	
c	*p*	*c*	*p*	*c*	*p*	*c*	*p*
113 ± 14	75 ± 14	75 ± 6·6	66 ± 6·6	124 ± 9·6	133 ± 8·4	113 ± 13	86 ± 11

c = control, *p* = with progesterone *in vitro*

Table II.–ATPase activity of rats injected with progesterone (10 mg for day)

Days of injection	Liver homogenates in H_2O		Liver homogenates in 0·25 *M* sucrose		Liver mitochondria in 0·25 *M* sucrose		Heart mitochondria in buffer borate	
	c	*p*	*c*	*p*	*c*	*p*	*c*	*p*
1	86	50	60	64	124	140	177	157
2	165	134	175	166	116	125	275	215
3	114	114	83	76	90	95	156	151
4	69	64	59	59	64	75	105	94
5	41	36	30	28	128	130	85	75

c = control, *p* = with progesterone *in vitro*

Table III.–ATPase activity of normal rats and injected with progesterone
(Livers and hearts were stored at 30°C)

Days of injection	Liver homogenates in H_2O		Liver homogenates in 0·25 *M* sucrose		Liver mitochondria in 0·25 *M* sucrose		Heart mitochondria in buffer borate	
	c	*p*	*c*	*p*	*c*	*p*	*c*	*p*
Normal	72	103	60	96	75	85	130	105
1	133	149	119	122	53	53	189	152
2	153	128	98	98	54	43	116	89
5	25	39	20	23	60	52	63	50

reaction was stopped by addition of 2 ml of cold 40% trichloroacetic acid. P determinations were made according to the method of FISKE and SUBBAROW[1], by reading the colour at 730 mμ in a BECKMAN Mod DU Spectrophotometer. Colour was developed with 40% $SnCl_2$ in concentrated hydrochloric acid. The N determinations were made by usual microkjeldhal tecnique. Progesterone was a crystalline preparation received from RICHTER[2].

When the influence of this substance on ATPase was tested, it was added as alcoholic solution and final concentration of the reaction mixture was 2×10^{-4} *M*.

In a first group of experiments, the influence of progesterone was tested on both water and 0·25 *M* sucrose liver homogenates and on liver and heart mitochondria. The results are represented in Table I, which shows that progesterone exerted a marked inhibiting influence on ATPase. ATPase activity was about 30% higher with water than with sucrose homogenates. This fact agrees with previous reports by KIELLEY and KIELLEY[3] and DIANZANI[4], who interpreted this fact due to the osmotic damage of mitochondria.

In another series of experiments, the effect of the administration of progesterone to living animals on the ATPase activity was studied. The rats received 10 mg progesterone daily for 1–5 days by intramuscular injection. The results are given in Table II under *c* = control indication. The action of progesterone *in vitro* was tested again on these same preparations (the results were shown under *p* = progesterone indication). It is clear from this table that ATPase activity increases strongly 2 days after the beginning of the treatment with progesterone and declines on subsequent days. The progesterone action *in vitro*, on preparations from animals treated *in vivo* with the hormone, agrees with results of Table I. In fact there was always a inhibitory effect on heart mitochondria, a small increase of liver mitochondria, an inhibitory action on liver homogenates prepared with 0·25 *M* sucrose or water. As in Table I, the values of the ATPase activity were always higher in water homogenates than in 0·25 *M* sucrose.

In a third group of experiments, the tissues of normal animals and animals treated with progesterone were stored at – 30°C for 24–48 h before use. Then the ATPase activity was determinated on homogenates and mitochondria. The results of Table III show that all ATPase values were lower than those for fresh tissues. Besides, only under these conditions was ATPase of liver homogenates increased *in vivo* and *in vitro* by progesterone, which agrees with JONES' and WADE's researches. On

[1] C. H. FISKE and Y. SUBBAROW, J. Biol. Chem. *66*, 375 (1925).
[2] Via Chioggia 2, Milano.
[3] W. W. KIELLEY and R. KIELLEY, J. Biol. Chem. *191*, 485 (1951).
[4] M. U. DIANZANI, Biochim. et Biophys. Acta (in press).

liver and heart mitochondria, on the other hand, progesterone always exerted a inhibitory action.

One may conclude from these experiments that the real influence of progesterone on ATPase activity of mitochondria is an inhibitory one. The fact that acceleration occurs with homogenates from livers stored at – 30°C, as JONES and WADE found and as these researches confirm, may depend upon a damaging of the mitochondria.

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Riassunto

L'autrice ha studiato l'influenza del progesterone sull'attività ATPasica di omogenati e mitocondri di fegato e cuore di ratto. Sui mitocondri di cuore il progesterone ha sempre azione inibente sia *in vivo* che *in vitro*. Sui mitocondri di fegato ha azione leggermente accelerante. Sugli omogenati di fegato preparati da tessuti freschi ha azione inibente. Se si conservano i tessuti a – 30°C si ha invece un aumento dell'ATPasi *in vitro*.

The Buffering Effect of Adrenal Tissue During Corticoid Overdosage

(Further studies on the physiological antagonism between gluco- and mineralo-corticoids)

The theory of stress and the diseases of adaptation is largely based upon the following assumptions: (1) that the adrenals can produce glucocorticoids (which, as far they have been examined uptodate, proved to be also antiphlogistic, lympholytic and catabolic) and mineralocorticoids (which, as far as they have been examined up todate, proved to be also prophlogistic, antilympholytic and anticatabolic) independently of each other, (2) that adjustments of corticoid secretion during stress is an important factor in regulating resistance, and (3) that derangements not only in the total amount, but also in the balance between these two types of corticoids is an important factor in the pathogenesis of various diseases[1].

The recent demonstration that cortisol[2] and aldosterone can be detected not only in adrenal tissue but also in the circulating blood has definitely rendered the "unitarian theory" of adrenocortical function untenable[3] yet, during the last four years, several investigators expressed doubts concerning the importance, or even the existence, of any true antagonism between mineralo- and gluco-corticoids. We have therefore devoted a great deal of attention to means of perfecting experimental techniques for the demonstration of such antagonisms, which to our mind are extremely important both in physiology and in pathology.

Experimental Procedure. Eighty Sprague-Dawley rats, having an average body-weight of 131 g (range 125–140 g) were subdivided into eight equal groups and treated as outlined in the Table.

Bilateral adrenalectomies were performed in Groups V–VIII, through the lumbar route and treatment with the steroids was begun on the same day. Microcrystal suspensions of cortisol acetate (250 µg/day) and DCA (1 mg/day) were administered in 0·2 ml of water, both steroids being injected subcutaneously in the ventral region. In the rats of all groups, granuloma-pouches were prepared two days later, under the shaved skin of the back, in the usual manner[1]. Under ether anesthesia, 25 ml of air was first injected, this being immediately followed by the introduction of 0·5 ml of an 0·5 % croton-oil solution (in corn-oil). The animals were maintained exclusively on "Purina Fox Chow" and tap water.

The formation of exudate was followed daily by trans-illumination with an electric flashlight and all surviving animals were killed 12 days after preparing the granuloma-pouch. Immediately after autopsy, the exudate in the granuloma-pouches was accurately measured by aspiration into a graduated syringe. At the same time, the adrenals, thymuses and spleens of all animals were dissected and fixed in Susa solution for subsequent weighing and histological study.

Experimental Observations. Our principal observations are summarized in the Table, so that it will suffice merely to mention the most salient features here.

It should be said at the outset that many preliminary experiments showed the impossibility of keeping alive, for 12 days, more than a few exceptional adrenalectomized rats with granuloma-pouches, if they received neither hormone treatment nor salt supplements. Hence, in the present experiment, only two rats of Group V survived so that here the means listed in the Table could not be accompanied by the standard errors (as was the case in the other seven groups in which all animals survived until the end of the experiment).

The *adrenals* in the intact-untreated rats (Group I) were of normal size, while those of the rats treated with cortisol, DCA or a combination of the two hormones showed varying degrees of the well known phenomenon of "compensatory atrophy". Thus, in this respect, the two types of steroids are not antagonistic and, indeed–as we have shown elsewhere[2]–at higher dose-levels, they are even synergistic.

In the intact rats, the formation of *exudate* under the influence of local croton-oil irritation, was only insignificantly affected by cortisol, DCA or the combination of the two agents, at the dose-levels used. Conversely, after adrenalectomy, cortisol alone proved to be clearly antiphlogistic (Group VI) and DCA prophlogistic (Group VII), while combined treatment with both hormones resulted in a mutual antagonism between them (Group VIII).

The two *lymphatic organs*, the thymus and the spleen, may be discussed conjointly, as they reacted essentially in the same manner to the two types of steroids. The lympholytic effect of cortisol was comparatively slight in intact animals, and correspondingly, the anti-lympholytic effect of DCA could likewise not become very obvious. Conversely, after adrenalectomy cortisol produced a marked thymolysis and splenic involution (Group IV), DCA had an inverse effect (Group VII) and, even more significantly, the effect of cortisol upon these lymphatic organs was very effectively counteracted by simultaneous treatment with DCA (Group VIII).

[1] H. SELYE, *Stress, The Physiology and Pathology of Exposure to Systemic Stress* (Acta Inc., Med. Publ., Montreal, 1950); *The Story of the Adaptation Syndrome* (Acta Inc., Med. Publ., Montreal, 1952).

[2] In agreement with the suggestion made by SHOPPEE [C. W. SHOPPEE, in: Ann. Rev. Biochem. *22*, 261 (1953)], the term cortisol is used here in preference to "hydrocortisone" since it eliminates confusion with 4,5-dihydrocortisone and obviates the possible implication that "hydrocortisone" is to be regarded primarily as a mere derivative of cortisone.

[3] S. A. SIMPSON and J. F. TAIT, in: Fourth Annual Report on Stress by H. SELYE and G. HEUSER (Acta Inc., Med. Publ., Montreal, 1954).

[1] H. SELYE, J. Amer. Med. Ass. *152*, 1207 (1953).

[2] H. SELYE, *Stress, The Physiology and Pathology of Exposure to Systemic Stress* (Acta Inc., Med. Publ., Montreal, 1950).