TWO MECHANISMS WHICH INCREASE *IN VIVO* THE LIVER TRYPTOPHAN PEROXIDASE ACTIVITY: SPECIFIC ENZYME ADAPTATION AND STIMULATION OF THE PITUITARY-ADRENAL SYSTEM.

W. E. KNOX.*

From the Molteno Institute, University of Cambridge.

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THE activity of the tryptophan peroxidase system (Knox and Mehler, 1950) in the livers of rats is greatly increased within a few hours of administration of tryptophan or of certain other compounds (Knox and Mehler, 1951). In the present paper it will be shown that this phenomenon is due to two independent mechanisms: (1) The increase in the enzyme activity following the administration of the substrate tryptophan is due to a mechanism resembling that of enzyme adaptation in micro-organisms. (2) The increase in the enzyme activity after administration of certain other substances which are not substrates of the enzyme system can be considered as a physiological adjustment characteristic of animals (Knox and Mehler, 1951), and is due to stimulation of the pituitary-adrenal system. The two mechanisms by which the enzyme activity can be increased, one adaptive and one hormonal, are of additional interest in revealing a potential means of regulating metabolism, not by affecting the enzyme reaction itself, but by altering the amount of the enzyme.

METHODS.

Hooded and albino rats of both sexes, weighing 180 to 220 g., were used. No strain or sex differences in enzyme activity were noted. The adrenalectomized animals were operated upon under ether anaesthesia, since nembutal treatment was found to depress the tryptophan peroxidase activity. They were then given 1 per cent NaCl as drinking water, and used on the third post-operative day. The completeness of adrenalectomy was verified by gross post-mortem examination.

The various compounds to be tested were injected intraperitoneally into both intact and adrenalectomized rats, as solutions or suspensions in 4 ml. water. The animals were killed five hours later, by which time full development of the enzyme increase had occurred (Knox and Mehler, 1951). Untreated animals were included in each group studied. The livers were removed promptly after death, cooled in ice, homogenized for 2 min. in a Waring Blendor with 8 volumes of cold 0·9 per cent KCl, and each immediately assayed.

Enzyme assay.

Two concentrations of each homogenate, 1·0 and 2·0 ml. (0·5 and 1·0 ml. of highly active homogenates) in 4·0 ml. total volume were assayed. Additions

* Special Research Fellow of the United States Public Health Service.

were 0·5 ml. 0·2 M phosphate, pH 7·0, and 0·3 ml. 0·03 M L-tryptophan (omitted from the blank run with each concentration). These mixtures were incubated aërobically with shaking at 38° for 1 hr., and the kynurenine formation determined, after Zn acetate-NaOH de-proteinization, by the increased absorption at 360 mμ. in a Beckman spectrophotometer.

Under these conditions L-tryptophan is oxidized to formyl-kynurenine by the two-step coupled oxidation reaction of the tryptophan peroxidase-oxidase system. The formyl-kynurenine is in turn hydrolyzed to kynurenine by the larger excess of formylase present in the liver homogenate. No added source of peroxide was necessary for the tryptophan peroxidase in these experiments, since the endogenous production of peroxide during the first two hours after preparation was adequate for full activity. The kynurenine formation was proportional to time up to several hours, and to the amount of the tryptophan peroxidase-oxidase system (Knox and Mehler, 1950). The activities determined with the two enzyme concentrations in each case were averaged, although the higher enzyme concentrations of the most active preparations were about 10 per cent less active than expected. This deficit in kynurenine accumulation was traced to a small activity of kynureninase (*cf.* Dalgliesh, Knox and Neuberger, 1951) which occurred at the higher kynurenine concentrations. This small further reaction of kynurenine tended to diminish rather than to exaggerate the increases in the tryptophan enzyme activity, so the error incurred has therefore been ignored.

RESULTS.

Action of other compounds compared to tryptophan.

The compounds which have been tested for their ability to increase substantially the tryptophan peroxidase activity in rat livers within five hours, including those previously reported on (Knox and Mehler, 1951) are listed in Table I, and arranged according to their effects on the enzyme level. The effective com-

TABLE I.—*The Levels of Activity of the Tryptophan Peroxidase-Oxidase System in Rat Liver after Administration of Various Compounds.*

Enzyme activity in liver after treatment.		
Normal.	Moderately increased. (approx. 2-fold).	Greatly increase (approx. 10-fold).
Na *L*-glutamate (6)	*DL*-phenylalanine (2)	*DL*-tryptophan* (11)
DL-alanine (2)	*L*-tyrosine (2)	*L*-tryptophan, 1 m.mol. (3)
Na acetate (2)	Na anthranilate (2)	
Sucrose (2)	Na gentisate (2)	
Ammonium chloride (2) (orally)	*L*-kynurenine* (1)	
	L-histidine-HCl* (12)	
	Histamine acid phosphate 0·005 m.mol. (4)	
	Epinephrine 0·001 m.mol. (6)	

* Levels produced by 2 m.mols of compounds so marked approximate the levels from 1 m.mol *L*-tryptophan.

L-tryptophan, epinephrine and histidine were given in the doses indicated, the other compounds in dose of 2 m.mols, by intraperitoneal injection to normal rats five hours before death. The number of animals given each compound is indicated in parentheses.

pounds other than tryptophan are: (1) inactive as substrates of the tryptophan peroxidase, (2) not necessarily related to each other or to tryptophan, (3) produce generally only double the normal level of enzyme in contrast with the ten-fold increases often produced by tryptophan, and (4) show wide differences in at least two cases in the dose necessary to produce the effect. A 1 m.mol dose of L-tryptophan, wehther it was given as such or as 2 m.mols of DL-tryptophan, increased the enzyme maximally. At least twice this dose of L-histidine or L-kynurenine (i.e., 2 m.mols of the L-isomer) was necessary to produce enzyme levels approaching those obtained with 1 m.mol of L-tryptophan. These compounds were accordingly classed as moderately effective. The other effective compounds only about doubled the normal enzyme activity. Adrenaline and histamine, however, produced this moderate increase with only about one-thousandth the dose necessary with the other compounds. These dosage differences, and the other differences listed above, suggested that the effective non-tryptophan compounds increased the enzyme by a separate mechanism from that of tryptophan itself.

Non-substrate compounds act through the pituitary-adrenal system.

A common mechanism by which the variety of non-substrate compounds could produce these similar increases was thought to be the relatively non-specific stress reaction. This reaction of the pituitary-adrenal system is well known to be stimulated by a wide variety of agents. Such stimulation could be expected simply from the large doses of the more insoluble compounds in themselves. Hyper-irritability and even coma were occasionally observed in the treated animals. Even some of the compounds listed in Table I as ineffective did irregularly cause moderate increases in enzyme activity when given to rabbits in massive doses. The stress reaction was specifically implicated by the increase in enzyme activity produced with small doses of adrenalin or histamine. Such doses of these two compounds to rats are known to cause adrenocorticotrophic hormone (ACTH) release and adrenal stimulation, reproducing the stress reaction (Long and Fry, 1945; Sayers and Sayers, 1948).

The role of the pituitary-adrenal system in producing the increased enzyme activity due to the moderately effective compounds was confirmed by studies of adrenalectomized animals (Table II). The levels of enzyme activity found in a series of untreated and treated intact rats have been tabulated in the upper half of the Table. The treated rats were given the most active non-tryptophan compounds: adrenaline and histamine, which act in very small doses, and histidine, which produces levels of enzyme activity approaching those due to tryptophan itself. A highly significant increase in the enzyme activity above the levels found in untreated animals was produced by each compound. In the lower half of Table II are given the levels of enzyme activity found after administration of these compounds to adrenalectomized rats. (Histamine was not tested in adrenalectomized rats). Some small effects may be noted, such as the possibly significant decrease in the enzyme activity in untreated animals due to adrenalectomy itself. But the most striking effect was the marked decrease or elimination after adrenalectomy of the usual enzyme responses to adrenaline and histidine. This result establishes the part played by the adrenal glands in increasing the liver tryptophan oxidizing activity after doses of the moderately effective, non-tryptophan compounds.

TABLE II.—*Comparison of the Activity of the Liver Tryptophan Peroxidase System Produced by Compounds Administered to Normal and to Adrenalectomized Rats.*

Enzyme activity (μmols kynurenine formed/g./hr.).

Compound given.	No treatment.	Epinephrine (0·2 mg.).	Histamine acid phosphate (2 mg.).	*L*-histidine HCl (0·31 g.).	*DL*-tryptophan (0·3 g.).
Normal rats	7·5	16·9	13·0	22·5	24·1
	8·4	23·2	15·6	36·4	27·0
	8·5	33·2	23·3	37·6	31·9
	8·4	27·2	..	35·9	51·9
	12·4	18·7	..	38·5	73·0
	10·6	25·6	..	44·2	96·2
	..	..	..	48·1	..
	..	..	..	50·2	..
Ave. ± σ	9·3±1·8	24·1±5·9	17·3±5·6	39·2±8·6	50·7±29·0
Adrenal-ectomized rats	6·3	6·4	..	6·4	25·5
	4·9	9·1	..	7·2	27·3
	7·8	3·2	..	9·2	42·0
	5·4	6·5	..	9·9	40·2
	3·7	15·7	..	10·3	35·9
	..	10·6	..	14·4	62·2
Ave. ± σ.	5·6±1·5	8·6±4·5		9·6±2·8	38·9±13·2

The rats were killed for enzyme assay five hours after intraperitoneal administration of the stated doses of the test compounds. The adrenalectomized animals given tryptophan died spontaneously in about 3½ hours, at which time the livers were promptly assayed. The enzyme activity given for each animal, in μmols kynurenine formed/g. dry wt. of liver/hr., is the mean of two determinations with different concentrations of each liver homogenate.

Action of tryptophan.

By the same type of experiment tryptophan was shown to act in a way different from that which involves the adrenal glands. The levels of enzyme induced by tryptophan administration to both intact and adrenalectomized rats are also given in Table II. The variable absorption of this insoluble amino acid within the time allotted produced a wide range of activities. Despite this variation, it can be seen that tryptophan produces large increases in the activity in intact rats and substantially as large increases also in adrenalectomized rats. The slightly lower levels in the adrenalectomized animals can be attributed to their spontaneous death about 4 hours after the dose of tryptophan, before the 5 to 6 hours necessary for full development of the enzyme increase. This exceptional toxicity of tryptophan to adrenalectomized animals is unexplained, although it does not prevent the adrenalectomized animals from developing amounts of enzyme after tryptophan administration comparable to those developed by intact animals. This response of the adrenalectomized animals to tryptophan, but not to the other compounds, clearly indicates the presence in animals of a specific mechanism which increases enzymic activity only after treatment with the substrate itself.

DISCUSSION.

The principal difference between the process in animals which results in increased tryptophan peroxidase and that in micro-organisms known as enzyme adaptation was previously pointed out to be the effectiveness of certain non-substrate compounds in increasing the animal enzyme (Knox and Mehler, 1951). These other compounds have now been shown to act separately from tryptophan, and the specific increases of the tryptophan peroxidase after tryptophan administration, as it occurs in adrenalectomized rats, can now be compared to the general process of enzyme adaptation.

Definition of enzyme adaptation in micro-organisms.

From various descriptions of the phenomenon (Dubos, 1940 ; Monod, 1947 ; Spiegelman, 1950) three characteristics have been selected which provide a conservative definition of enzyme adaptation. It should be emphasized that even in the field of microbiology these three characteristics have not necessarily all been demonstrated in each case accepted as enzyme adaptation.

1. A valid increase in the activity of a particular enzyme : Apparent increases, due to permeability changes or co-factor augmentation for example, are excluded by demonstration of the increased activity also in cell-free preparations and under the optimal conditions for the reaction.

2. The increased activity is produced by treatment of the cells with the enzyme's substrate or a very closely related compound. The mutual specificity of the compound inducing the adaptation and the enzyme it calls forth, a relationship like that of enzymes and their substrates (Mirick, 1943), is a positive characteristic of the phenomenon.

3. The increased activity is not referable to an increase in the mass of cells or to the selection of special cell types. The cells must be able to carry out synthetic reactions to adapt (Spiegelman, Kamen and Sussman, 1948), but need not grow (Stephenson and Stickland, 1933 ; Pollock, 1946 ; Brandt, Freeman and Svenson, 1951).

Characteristics of the increase in the tryptophan peroxidase.

Validity of the increase.—The high activity of the tryptophan peroxidase after treatment was previously demonstrated in liver slices and in cell-free preparations under the optimal conditions of the assay. The increased activity could not be attributed to permeability changes, provision of co-factors, reversal of inhibition, etc., and was accepted as a valid increase in activity, probably due to an actual increase in the enzyme concentration (Knox and Mehler, 1951).

Specific relation of enzyme to adapting compound.—The production of the increased activity by the substrate of the enzyme, tryptophan, and not by unrelated compounds, has here been demonstrated in adrenalectomized animals. In intact animals both this adaptive process and a second process involving the hormonal system can occur.

Independence from growth.—The increase in the tryptophan enzyme in liver by growth of a particular cell-type rich in this enzyme is reasonably precluded by the ten-fold magnitude of the activity change occurring within six hours. The generation time (for doubling) of fibroblasts in tissue cultures is about 24

to 48 hours (Fischer, 1946), and must be even longer for more highly differentiated cells *in vivo*. The rapid decrease to normal activity within eighteen hours as the given tryptophan is utilized (Knox and Mehler, 1951) is additional evidence that the change is not of the relatively permanent kind resulting from growth of new cells. Similar rapid losses of adaptive enzymes upon withdrawal from the substrates commonly occur with micro-organisms (Dubos, 1940; Spiegelman, 1946; Mirick, 1943). Moreover, isolated liver slices from untreated animals, incubated in oxygen, glucose and tryptophan, did not increase their tryptophan oxidizing activity. The synthetic abilities of liver are relatively poor under these conditions, and the failure to adapt, may be compared to the similar inability of dinitrophenol-treated yeast cells to adapt, in each case the adaptation being prevented by the lack of synthetic reactions (Spiegelman *et al.*, 1948).

From these considerations the increased tryptophan peroxidase produced by the administration of tryptophan can be accepted as an example of enzyme adaptation occurring in animals.

General adaptive mechanisms in animals.

There is a wealth of phenomena in animals which possibly includes other examples of enzyme adaptation (see also Bodansky, 1948). Among these are variations in the activities of arginase (Lightbody and Kleinman, 1939), β-glucuronidase (Fishman, 1940), pancreatic amylase and trypsin (Grossman, Greengard and Ivy, 1942), kidney glutaminase (Davies and Yudkin, 1951), and succinic dehydrogenase (Shipley, Meyer, Copenharer and McShan, 1950). In none of these cases have the validity of the increased activity, the close relationship between the compound and the adapting enzyme, and the independence of the increase from cell growth or replacement all been demonstrated. None can yet be accepted as enzyme adaptation on the basis of the criteria used above. Further work on the arginase and β-glucuronidase has indeed shown these changes in activities to be referable to other processes (Miller, 1950; Kerr, Campbell and Levvy, 1949). But with the identification of one example of enzyme adaptation in animals, the likelihood is increased that other similar changes are due to the same cause.

It is necessary, however, to distinguish between those enzymic changes due to enzyme adaptation, as a particular process, and those due to the other mechanisms which contribute to the general adaptability of living systems (growth, heredity and the varied processes accounting for the responsiveness of protoplasm). The criteria of enzyme adaptation used here are purposely conservative in order to minimize the confusion of this poorly understood process with the others. Among such other processes from which enzyme adaptation must be distinguished in animals is the action of hormones.

The hormonal mechanism increasing the tryptophan peroxidase.

The second mechanism which produces increased activity of the tryptophan enzyme was identified as an effect of the pituitary-adrenal system by its initiation with small doses of adrenalin or histamine, or with larger doses of possibly injurious compounds, and by its elimination upon adrenalectomy. ACTH is released from the pituitary by certain specific and many non-specific agents, including adrenalin and histamine, with consequent stimulation of the adrenal cortex and

production of the so-called stress or alarm reaction. The enzyme would then appear to increase in response to the adrenal hormones, a process distinct from its adaptive increase in response to the substrate. The effective compounds other than adrenalin and histamine may act by causing ACTH release (*cf.* Blanchard, Dearborn and Marshall, 1951), or only after their metabolic conversion to active amines (e.g. histidine to histamine).

The variety of effects following adrenal cortical stimulation is also called the general adaptation syndrome (Selye, 1946), which despite the similarity of names should not be confused with enzyme adaptation. One burden of the present work is to show that these are separate processes, which in this case share a common ultimate effect—an increase in the amount of the same enzyme. It is of interest that the increase in the tryptophan peroxidase develops in a few hours, and earlier than other objective effects of the stress reaction or of ACTH administration (White, 1949).

Changes in enzyme concentration as a process regulating metabolism.

The marked fitness between the stimulus and the response in enzyme adaptation suggests the possible function of this process. Enzyme adaptation can proportion the amount of an enzyme to the amount of its substrate, and in this way adjust the metabolism for the work in hand. The metabolism of L-tryptophan in animals, leading ultimately to the formation of nicotinic acid, is initiated by the tryptophan peroxidase and may be controlled by the amount of this enzyme. Since a very large change in the enzyme was produced by supplementary tryptophan equal to about twice the daily intake, a metabolically significant change could also be expected from the small fluctuations of the dietary tryptophan.

The metabolic regulatory mechanisms studied in animals up to the present have been largely hormonal. In general, the hormones have rarely been found to affect appropriate cell-free enzyme reactions directly, although in intact animals they obviously affect the metabolism utilizing these reactions. The separate processes of enzyme adaptation and of hormonal action, in the present study, both had the effect of increasing the amount of an enzyme *in vivo*. The production of a potential increase in metabolism by increasing the amount of enzyme, but without affecting the catalytic activity of a given amount of enzyme may therefore be a general means of metabolic regulation. Action of hormones by changing the amounts of enzymes concerned would account for the modifications of enzyme concentrations characteristic of many states of endocrine disbalance (Dempsey, 1946). And since changes in the amounts of the enzymes can only be produced by certain synthetic functions of the cell, the action of the hormones would understandably be not readily demonstrated in cell-free preparations.

SUMMARY.

Two mechanisms producing an increased amount of the liver tryptophan peroxidase in animals are described: the first produces large increases and acts only in response to the administration of the substrate, tryptophan; the second produces only moderate enzyme increases and acts in response to a variety of compounds, including adrenalin and histamine. These mechanisms both act in the normal animal, but adrenalectomy eliminates the second, the less specific and less effective process. The first mechanism has been identified as that of

enzyme adaptation, previously known only in micro-organisms, and the second has been identified as the stress reaction, acting through the adrenal glands.

The criteria of enzyme adaptation in micro-organisms on which the identification of this process in animals is based are: (1) a valid increase in enzyme activity; (2) which is produced by treatment with the substrate of the enzyme; and (3) which occurs independently of growth or selection of cells. It is also necessary in mammals to differentiate other processes which can alter enzyme activity, like the hormonal effects of the stress reaction acting through the adrenal glands in the present example.

These results suggest that alterations in the quantities of enzymes may be a general means by which metabolism is regulated.

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