FIG. 2

Steinhardt determines the position of the points A and B (constant Y) by the corresponding coordinates of the theoretical curves when fitted to the experimental points by an appropriate choice of the constants K′ and k. Since Figs. 1 and 2 involve a log *vs.* log plot, this can be accomplished graphically by rectangular transposition. Thus he determines $\triangle\, p_H$ for constant log k, corresponding to the horizontal component DB, by observing the p_H in the fitted data which the point on the theoretical curve for log K′ − log $a_H = 0$ falls. This p_H is identical with p_K.

Since

$$\triangle\, p_H = \triangle\, p_K = \log\, {}^{K}25/_{K_{15}} = 0.23,$$

$$2.3\ RT_2T_1\frac{\triangle\, p_H}{\triangle\, T} = 9{,}040\ \text{cal.}$$

corresponding to the heat of dissociation of a single proton. Five times this quantity (45,200 cal.) is $\triangle\, H$ for Step I.

Steinhardt's procedure is thus identical with that given above, which will be valid for the linear portion where practically all the pepsin is present as p^n. The simple procedure of setting $AD = AC - DC$, however, will not be valid for the less acid non-linear portion, designated by primes in Fig. 2, where a significant fraction of the pepsin exists as P^{n-5}. On the other hand the procedure of subtracting $\triangle\, H$, computed from $2.3\ RT_2T_1\dfrac{\triangle\, p_H}{\triangle\, T}$ is valid for both the linear and the non-linear portions.

When the true energy of activation, 18,300 cal., is employed in eq. 1, the product v s= $10^{9.3}$. Hence s lies between $10^{-2.1}$ and $10^{-3.5}$ depending upon whether one assumes $v = Z$ or k^T/h. These are reasonable values for the steric factor or true entropy of activation, and the kinetic paradox disappears.

SUMMARY

The abnormally large values of the energy and the entropy of activation encountered in protein denaturations and enzyme destructions are illusory, since the customary method of calculating these quantities by the comparison of rates at constant p_H alone is fallacious. This procedure includes, in addition to the true energy of activation, the heat of dissociation of all acidic equilibria involved in preparing the initial molecules for the kinetic step of activation. Steinhardt has shown by a simple and concrete mechanism that the kinetics of pepsin destruction obey the simple laws of chemical kinetics when correction is made for the preliminary acidic dissociation equilibria which are not part of the activation process.

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NICOTINIC ACID AND VITAMIN B₂

IN collaboration with Dr. Y. Subbarow, of the Department of Biochemistry, Harvard Medical School, I have been attempting to isolate and identify the accessory factors responsible for the prevention of the various diseases which have been attributed to a deficiency of vitamin B_2. Using a crude aqueous extract of liver as raw material, Dr. Subbarow has carried out the chemical fractionations, and biological tests have been made here. Evidence has been obtained which adds one more member to the group of factors comprising vitamin B_2.

Koehn and Elvehjem[1] described a fraction prepared from aqueous extract of liver and showed that it possessed curative properties for chick dermatitis and also for blacktongue in dogs, and concluded from this that the chick dermatitis preventive and blacktongue preventive factors were probably identical. Later, Elvehjem, Madden, Strong and Woolley[2] reported that nicotinic acid cured blacktongue in four dogs. In April of this year we isolated nicotinic acid from a highly purified fraction prepared from liver extract, and later obtained the related alkaloid trigonelline. We had already tested the nicotinic acid for curative activity in chick dermatitis and in rat dermatitis when the publication of Elvehjem *et al.* appeared. When incorporated in the basal diet for chicks described by Lepkovsky and Jukes[3] at levels up to 0.05 per cent. it

[1] *Jour. Biol. Chem.*, 118: 693, 1936.
[2] *Jour. Am. Chem. Soc.*, 58: 1767, 1937.

failed to protect the chicks against dermatitis and did not restore growth; when given to rats which had developed acrodynia on a vitamin B_6 deficient diet in daily doses of 1 mg or of 2 mg it failed to bring about a cure or restore growth. Following the publication of Elvehjem et al., nicotinic acid has been tried in blacktongue and has been found to cure the mouth symptoms and restore appetite and weight. Although further tests must be made to determine whether the blacktongue preventive activity of liver extracts is due to nicotinic acid alone, the claim of Elvehjem et al., that nicotinic acid cures blacktongue has been substantially confirmed. Thus direct test has shown that nicotinic acid prevents blacktongue but not chick dermatitis, proving that deficiencies of different factors underlie these two diseases, contrary to the conclusion of Koehn and Elvehjem.

As a result of this finding it now appears that under the heading of vitamin B_2 as defined after its separation from vitamin B_1 four factors are grouped if the human pellagra-preventive factor should be identical with the blacktongue preventive factor, or five factors if these two should prove to be distinct. Evidence of their identity is circumstantial, of the type which in the past led to belief in the identity of other pairs of these factors until further chemical purification led to a separation. Only the cure of both diseases by one and the same crystalline material can prove finally that they are caused by deficiency of a common factor.

Assuming that the blacktongue preventive and pellagra preventive factors are identical, it appears that vitamin B_2 comprises four entities. Two of these, flavin and nicotinic acid, have been isolated and identified. Two more have been distinguished but not isolated: these are the vitamin B_6 (preventive of rat dermatitis) and the factor preventive of chick dermatitis. Neither of these is identical with flavin, as shown by the work of György[4] and of Koehn and Elvehjem,[5] respectively. Our observations now show that neither is identical with nicotinic acid. If clinical tests show that nicotinic acid is pellagra-preventive, the list will be complete; but if it is not, the pellagra preventive factor must be added, making a total of five.

On account of its supposed identity with the pellagra-preventive factor, Elvehjem and Koehn have used the name vitamin B_2 to denote the chick pellagra-preventive factor. Since we have shown these factors to be distinct, there remains no single reason to support this usage, and in view of early definitions the name vitamin B_2 should be used to denote the complex made up of all the factors mentioned above. If it is used for any single factor, then it should be reserved for the human pellagra preventive factor.

A detailed report of our observations will be published elsewhere.

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SPONTANEOUS LEPROSY IN A MOUSE

A SPONTANEOUS leprosy-like disease in rats is very well known. It was first described by Stefansky[1] in 1903 at Odessa and almost simultaneously and independently by Dean[2] in England. Subsequent reports by various authors have established its wide-spread distribution in different parts of the world.

Two varieties of the spontaneous disease have been described, the musculocutaneous and glandular. In the former, there is extensive involvement of the skin, subcutaneous tissues and skeletal muscles with associated alopecia, ulceration and loss of subcutaneous fat. In the other form there is extensive lymphatic glandular involvement, both focal and more universal, but chiefly confined to those of the axilla, groin, neck and submaxillary regions. This division is, however, not very strict. The lymphatic glands are involved in the cutaneous form and the skin to a mild degree in the glandular type. In both there is visceral involvement as well.

A similar disease in the closely related mouse has hitherto not been described.

A brown wild house mouse (mus musculus) was found wandering through our laboratory in broad daylight. It was evidently deformed, sickly in appearance and could readily be caught. It presented all the features of the musculocutaneous variety of the disease, as seen in the rat. There were alopecia and thickening of the skin of the scalp with distortion of the pinnae and patchy areas of alopecia over the skin of the back with two discrete gray circular ulcers, measuring on the average 0.15 cm in diameter. The anterior pubic and perineal regions were enormously thickened and prominent. The base of the tail was in consequence deviated to the right. Both hind limbs, particularly in the femoral and tibial regions, were likewise greatly thickened and nodular. The forelimbs were involved, but to a lesser degree. Sections through the skin revealed diffuse infiltration of the subcutis by softish yellowish-white tissue. In the scalp both corium and subcutis down to calvarium were thus infiltrated and thickened. In the forelimbs the corium was less involved; the subcutis, however, was quite markedly thickened and infiltrated. In the hind limbs, there was minimal involvement of the corium which could readily be stripped from the underlying, greatly infiltrated, tissues. The cutis, subcutis and all struc-

3 Jour. Biol. Chem., 114: 109, 1936.
4 Biochem. Jour., 29: 745, 1935.
5 Jour. Biol. Chem., 108: 709, 1935.

1 W. K. Stefansky, Centralblatt f. Bakt. und Parasitenkunde, 33: 481, 1903.
2 G. Dean, Centralblatt f. Bakt. und Parasitenkunde, 34: 222, 1903.