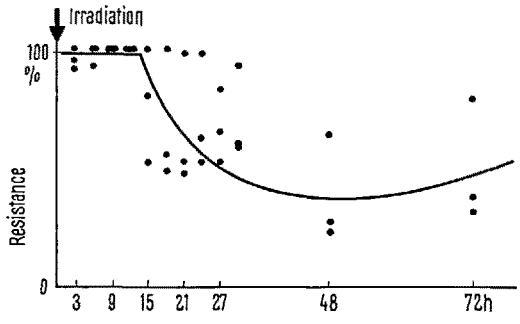

Fig. 1. The level of chlortetracycline-induced resistance to tetracyclines of faecal coliforms of conventional piglets before and after 600 r ($\simeq LD_{50/30}$) of total-body X-irradiation in days.

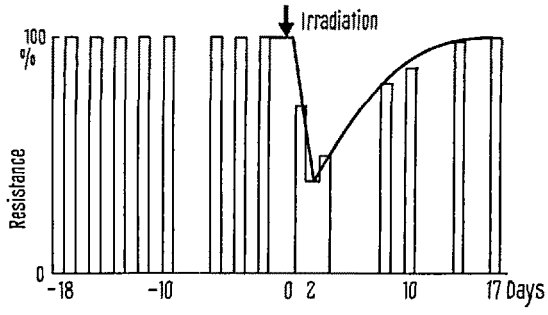

Fig. 2. The level of chlortetracycline-induced resistance to tetracyclines of faecal coliforms of 3 conventional piglets after 600 r total-body X-irradiation in hours.

and afterwards 48 and 72 h, and 8, 10, 14 and 17 days after exposure (Figures 1 and 2).

During the whole period before irradiation until 12 h after irradiation the resistance was practically equal to 100%. The first signs of post-irradiation resistance depression were detected in 2 piglets 15 h and in the third piglet 21 h after exposure. The mean values of resistance in days and hours are given in the Figures.

After irradiation, a latent period of 12–15 h ensues, after which the resistance decreases, reaching the lowest level about 48 h after exposure. (In our previous experiments[1] the 48-h-interval was omitted, and the minimum seemed to be in the 24th h following irradiation.) After an interval of 72 h the first signs of gradual rise again can be detected. The resistance reaches its normal on about the 17th day (according to previous experiments, on the 20th day).

It can be concluded that the onset of the post-irradiation depression of CTC-induced resistance of coliform faecal organisms begins about 15 h after exposure, the resistance reaches the minimal level 24–48 h afterwards and returns to the original level about 17–20 days post-irradiation.

If we take into account that one generation of coliform microbes takes 18–30 min, the number of 30–50 generations alternates in the host from the 'hit' of irradiation to the moment when its first effects on intestinal bacteria appear. However, this 'hit' is long enough, taking 50 min for each animal. It signifies that the supposed genetic information, resulting in the 'mass' effect of resistance depression, should be conserved in and transmitted by 30–50 generations. The other 66–110 generations succeed till the phenomenon of resistance depression reaches its maximum, i.e. manifests itself in 60% of individuals of the coliform population (see the fall of resistance from 100 to 40%).

Nevertheless, it must be mentioned that the method used, revealing the properties of coliform component in total (about 2.5×10^7 individuals/g of rectal content), does not allow the study of the latent phase of these changes, nor does it evaluate the degree of resistance quantitatively.

Résumé. Chez des porcelets totalement irradiés par une dose de $\cong LD_{50/30}$ de rayons X, la résistance de la microflore choliforme fécale provoquée par de faibles doses de chlortétracycline commence à céder 15 h après l'exposition.

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and P. Otoupal

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Dissociation of Urease in Acetate Buffer of pH 3.5 with Retention of the Enzymatic Activity

A molecular weight of 483,000 was calculated for urease in phosphate buffer of pH 7 from its sedimentation and diffusion coefficients (s^0 18.6 S; D 3.46×10^{-7}; $\bar{v}$ 0.730)[1]; recently, this value has been at least approximately confirmed by equilibrium ultracentrifugation measurements, despite problems caused by polydispersivity[2]. This communication reports that on treatment with 0.1 M acetate of pH 3.5 urease dissociates within 1 h or less to a weight of 240,000 and that this dissociated product is enzymatically active; its activity in pH 3.5-acetate is 40% as great as that in 0.34 M phosphate of pH 7[3]. The acetate medium causes irreversible denaturation, but at a rate much slower than that of dissociation. A preliminary report of this work has been presented[4].

Urease was isolated as described by Mamiya and Gorin[5] (no mercaptoethanol employed) and recrystallized 3 times. The preparations had specific activities of 1600 to 1850 U_a^{25}/mg (equivalent to 145–171 Sumner units)[3,6]. Figures 1 (a) and 1 (b) contrast the sedimentation velocity of urease that was dissolved, respectively, in 0.02 M phosphate of pH 7 and in 0.1 M acetate of pH 3.5 (note

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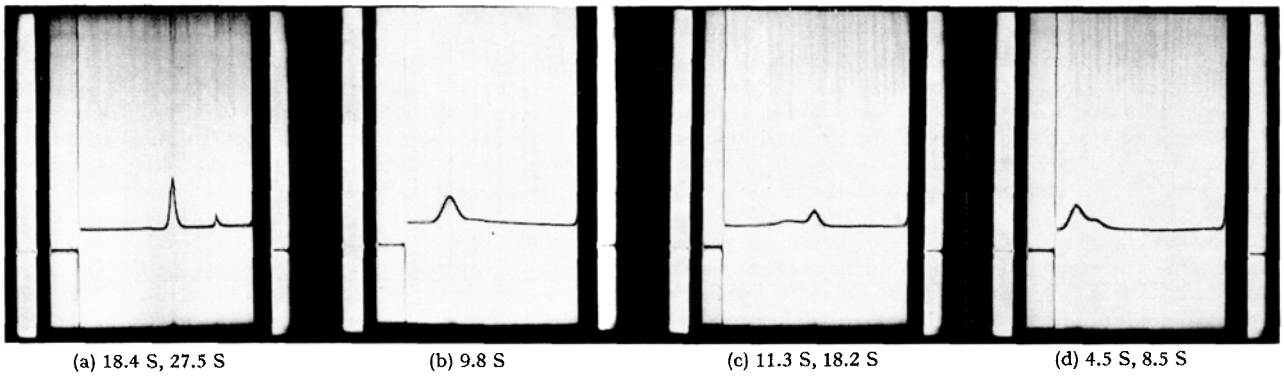

(a) 18.4 S, 27.5 S (b) 9.8 S (c) 11.3 S, 18.2 S (d) 4.5 S, 8.5 S

Fig. 1. Sedimentation patterns of urease solutions, (a, b, d) ca. 5 mg/ml. All pictures taken at 59,780 rpm, 20 min after attaining speed, with 75° schlieren angle: (a) in 0.02 M phosphate, pH 7; (b) in 0.1 M acetate, pH 3.5, 1 h after preparation of solution; (c) aliquot of (b) readjusted to 7; (d) in 0.1 M acetate, pH 3.1, 1 h after preparation of solution.

that pH and concentrations must be controlled closely; the solution was prepared by dissolving urease in triply-distilled water and mixing it with an equal volume of buffer consisting of 53.3 ml 0.02 M acetic acid + 3.7 ml 0.02 M sodium acetate). Pattern 1 (a) did not change appreciably over a 24-h period. Pattern 1 (b) was determined at 1 h after mixing; subsequent determinations at intervals over a 48-h period showed no qualitative change, but the s-value decreased slowly to 8.0 S.

Urease is catalytically active at pH 3.5. To determine the activity, the enzyme solution was diluted some 200-fold with buffer, 1 ml aliquot was mixed with 1 ml of 3% urea in buffer, the reaction was stopped after 2 min with 1 ml of 1 M H_2SO_4, and the NH_3 was determined with Nessler's reagent. Assays were also conducted in SUMNER's substrate of pH 7[6], with a 2-min reaction period. No ethylenedinitrilotetraacetate was added to the buffers[3,6] because it inhibits the reaction at pH 3.5; assays were conducted immediately after dilution. In pH-7 phosphate, the activity remained essentially unchanged for at least 24 h (< 5% decrease). If a solution of enzyme was made up in pH-3.5 buffer and assayed at pH 7 within 10 min the activity found was the same as for urease that had been dissolved at pH 7. The activity in pH-3.5 acetate immediately after preparation of the solution was 40% of that exhibited at pH 7. With time, the activity of the enzyme in pH-3.5 buffer decreased as shown in Figure 2; the same degree of inactivation was found by assaying at pH 7 and at 3.5. Although some variation in rate was observed in different experiments, especially after 8–24 h, the activity after 1 h was never less than 70% of the initial value; i.e., the species having an s-value of 9.8 retained a large proportion of the initial activity.

In order to estimate its weight, it is necessary to ascertain whether a change in frictional ratio had taken place. This may be assessed from the change in specific viscosity, also shown in Figure 2. It can be seen, first of all, that the initial value is quite comparable to that of other globular, symmetrical proteins; this indicates that native urease has a compact, nearly spherical structure. Over a period of hours, the viscosity increases substantially and apparently reaches a limiting value; this may reasonably be ascribed to disruption of the native structure to give a more asymmetric, less compact product. In 1 h, however, the increase in viscosity is only some 20% of the total change, and most of it must be due to the formation of denatured enzyme. It follows that the remainder of the material must have a frictional ratio near that of native urease. If there had been no change in

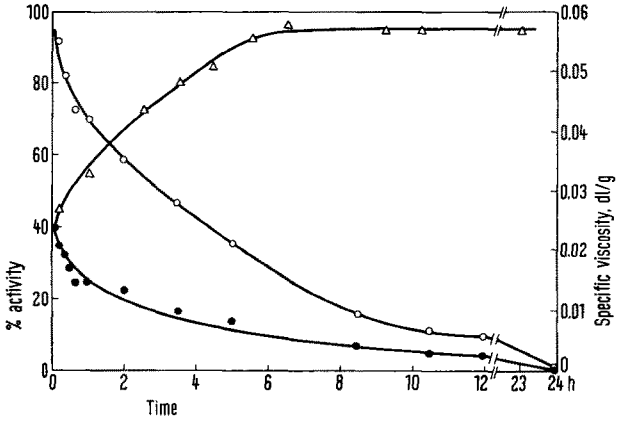

Fig. 2. Properties of urease solution, 5 mg/ml, in 0.1 M acetate, pH 3.5, at 25°. Left ordinate, circles: % activity remaining; empty circles, assayed in SUMNER's substrate; full circles, assayed in pH 3.5. Right ordinate, triangles: specific viscosity.

frictional ratio, the following relation would hold: $M_1/M_2 = (s_1/s_2)^{2/3}$; this gives M = 190,000, which does not correspond to an even splitting of the 480,000-unit. However, one would expect the aforementioned result to be low, for the following reasons: (1) the s-value presumably refers to a mixture of active and denatured material, that is not resolved into separate peaks in the conditions; (2) the s-value is for a finite concentration, and s^0 would likely be somewhat higher; (3) the frictional ratio may in fact be somewhat greater than that of undissociated urease. Although we cannot make quantitative estimates of the corrections, we believe one can reasonably deduce that the 480,000-unit is split into halves.

Treatment of urease with 6 M guanidinium chloride[7] or with sodium dodecyl sulfate[8] causes dissociation into subunits of weight 60,000–80,000. It appears, therefore, that the native 'molecule' is an aggregate of 6–8 subunits. We conclude from the results described that in pH 3.5 this state of aggregation becomes unstable and that there

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occurs, first, a rapid dissociation into halves. In this time, there is little loss of activity and we suppose that there can be little alteration of the subunit structure. Disruption of the subunit structure does take place, but much more slowly, as exposure to pH 3.5 is prolonged. Since the value of *s* decreases only from 9.0–8.0 in the period from 1–48 h, we also conclude that disruption of the subunit structure does not cause further dissociation.

Figure 1 (c) shows that the change from 18–9.8 S could be largely reversed, after 1 h. After longer exposure to pH 3.5, the extent of regeneration of the 18-S peak decreased, with a concomitant increase in material of 8–10 S; e.g. in an aliquot portion that was adjusted to pH 7 after 6 h at pH 3.5, the area of the 18-S peak amounted to about $^1/_3$ of the total. Although there is a qualitative correspondence between the decrease in activity, the increase in viscosity, and the loss of the ability to reaggregate, these do not correspond quantitatively (cf. Figure 2). We believe this may be due to the fact that in the progress toward complete denaturation there are formed diverse 'mixed' aggregates, that contain both denatured and native subunits. This makes it impossible to interpret the data in terms of a simple model.

Many experiments were done at other concentrations and pH values, that will not be reported for lack of space. In general, they show that both pH and concentration influence the dissociation and denaturation processes. Some indicative results are (all 1 h after preparation of the solution): (1) 0.1 *M* acetate, pH 4.2, caused little dissociation; (2) 0.02 *M* acetate, pH 3.5, induced substantial but not complete dissociation to a 10-S product; (3) 0.1 *M* acetate buffer of pH 3.1 produced lower-*s* products, as exemplified in Figure 1 (d), and the activity was lost very quickly.

There have been some earlier reports in the literature concerning the existence of urease with an *s*-value of 8–12[9-11]; however, the characterization of the products mentioned in these reports is not sufficiently detailed to permit a meaningful comparison with the 10-S product described in this communication. Very recently, BLATTLER et al.[12] have reported that urease exposed to *Tris* buffer of pH 9 and 90% 1,2-propanediol is dissociated into halves and that the activity retained. It seems quite probable that this product is closely related to that obtained in the present work[13].

Riassunto. Le molecole dell'urease (peso molecolare 480,000, coefficiente di sedimentazione 18,6 S) si dividono a metà quando trattate con tampone acetato, 0,1 *M*, pH 3,5. Inizialmente, l'attività enzimatica è 40% di quella al pH 7 (tampone fosfato, 0,34 *M*) e la dissociazione è riversibile. Prolungata esposizione al pH 3,5 causa una lenta denaturazione, con perdita dell'attività e dell'abilità di ricostituirsi.

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26 February 1968.

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Effect on the Monoamine-Metabolism of the Mouse Brain by Experimental *Herpes simplex* Infection

The syndrome observed after administration of the dopamine precursor L-DOPA is characterized by excitation and a pronounced peripheral sympathetic tonus. The dopamine (DA) concentration will reach very high values in the corpus striatum area. Mice infected i.c. with *Herpes simplex* virus reveal during the course of infection i.a. signs of excitation, similar to those observed after administration of L-DOPA. These symptoms precede the more severe symptoms of encephalitis, such as convulsions.

Abnormal low concentrations of dopamine are found in the neostriatum and substantia nigra areas in post mortem examinations of patients with Parkinson's disease[1]. A decrease in the concentration of 5-hydroxy-tryptamine (5-HT)[2] has also been demonstrated in the brains of such patients. It is known that Parkinson's disease might appear after time periods of varying length following an acute encephalitis. Against this background we considered it important to study experimentally the possible relationship between virus induced encephalitides and the monoamine-metabolism of the brain.

Swiss albino mice of our own laboratory breed were inoculated i.c. with a mouse-brain-adapted strain (St2Gbg 10) of *H. simplex* virus. A series of experiments were performed in which the amount of virus inoculated (30–300 LD_{50}) was adjusted to produce encephalitis but not to kill the mice during the first 5 days after the inoculation. On day 5 after inoculation, when most of the mice showed signs of encephalitis (ticks, convulsions or lethargia) 15–22 mice were selected, sacrificed by ether narcosis, and the brains were analysed for the contents of DA and homovanillic acid (HVA). In parallel, the brains of mice inoculated with an isotonic NaCl solution or brains of non-inoculated mice were analysed. As in the first 4 experiments, no differences were observed if non-inoculated mice or mice given the NaCl solution were used; only non-inoculated mice were used as controls in following experiments.

Determinations of DA and HVA were made spectrophotofluorometrically according to the method described by CARLSSON and WALDECK[3] and that of ANDÉN et al.[4].

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