every day of incubation. The protein content of the preparations was also determined[9].

In control liver the hydroxylase activity was negligible throughout the experimental period; all the average values of the specific activity (mμmoles of tyrosine formed/h/mg protein) at each incubation day were between 8 and 20, without notable change immediately after hatching. Similarly, the dehydrogenase level in the pre-hatching period was very low, averages of the specific activity (mμmoles of NADH formed/min/mg protein) being between 25 and 41. This enzyme, however, increased immediately after hatching to 161, 4 times that of the previous day level. In the case of each enzyme, neither dose group of the irradiated liver showed any meaningful, statistically significant change in activity from that of the control. The result was also inconclusive in the case of hydroxylase with a single injection of 2.5 μg cortisone in saline into the air chamber of the eggs immediately after or before the irradiation, at several stages of incubation. A dose of 400 r was not successful in enticing a change in the dehydrogenase activity.

The developmental pattern of the hydroxylase in the chick embryo seems to be similar to that of mammals, including the human[1,3], although a more positive view has been expressed[10]. The sudden increase of the dehydrogenase confirms previous work[4] and is comparable to that of other mammalian enzymes[11,12]. The low conversion rate of phenylalanine to tyrosine in the new-born rat was attributed to the low content of the co-factors[13], and injection of molybdenum enhanced the dehydrogenase activity in the chick embryo[4]. Although both enzymes are co-enzyme-dependent, the pyridine nucleotides were not an effective stimulator[14]. Despite much emphasis on the endocrine functions in the adult, several lines of experimental evidence indicate that factors such as the initial level and the rate of formation of the enzyme could play a more significant role in embryonic enzyme induction: (1) contrary to the condition in the adult, substrate injection did not activate the fetal liver enzyme[11], and this appears to be true in the chick embryo[4]; (2) the sudden increase of enzyme in the chick embryo[4] or in mammalian liver[15] was completely blocked by inhibiting protein synthesis; (3) many enzymes are influenced by age, sex, and nutrition, in addition to hormones[2,12,16]. Therefore, the lack of enzyme activation in this experiment could be attributed to these general conditions.

Résumé. Bien que l'activité de l'hydroxylase phenylalanine et de la déhydrogénase hypoxanthine dans l'embryon de poulet ne soit pas évidente, la déhydrogénase hypoxanthine augmente d'une façon rapide justement après l'éclosion. L'induction des enzymes par l'irradiation par les Rayons X n'est pas effective non plus.

U. K. HWANG[17]

Biology Division, Atomic Energy Research Institute, Seoul (Korea),
5 December 1967.

[9] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[10] W. L. RYAN and W. ORR, Archs Biochem. Biophys. *113*, 684 (1966).
[11] A. M. NEMETH, J. biol. Chem. *234*, 2921 (1959).
[12] F. SERENI, F. T. KENNEY and N. KRETCHMER, J. biol. Chem. *234*, 609 (1959).
[13] A. R. BRENNEMAN and S. KAUFMAN, J. biol. Chem. *240*, 3617 (1965).
[14] G. H. REEM and N. KRETCHMER, Proc. Soc. exp. Biol. Med. *96*, 458 (1957).
[15] A. M. NEMETH and G. DE LA HABA, J. biol. Chem. *237*, 1190 (1962).
[16] G. WEBER, G. BANERJEE and S. B. BRONSTEIN, Am. J. Physiol. *202*, 137 (1962).
[17] Dept. of Anatomy, St. Louis University, St. Louis, Missouri 63104, USA.

Onset of Post-Irradiation Depression of Chlortetracycline-Induced Resistance of Faecal Coliforms in Young Conventional Pigs

The coliform component of faecal microflora of conventional piglets, which were maintained for 1–2 weeks after weaning on a diet supplemented with chlortetracycline (CTC) (daily dose $\simeq$ 10 mg/kg body weight) includes a high percentage – practically 100% – of strains resistant to tetracyclines. We demonstrated a considerable temporary depression of this resistance after total-body sublethal and half-lethal doses of X-irradiation, when the piglets – after being 'prepared' by low CTC doses for a short period – were fed a diet without a supplementary dose of CTC during the whole experiment[1].

The mechanism of the effect of these doses of ionizing radiation (550 and 600 r), which are insufficient for any direct influence on bacteria[2], is not known. We assume that the macroorganism damaged by the radiation or, more accurately, its intestinal tract which has a relatively high degree of radiosensitivity and which represents the life environment for intestinal microorganisms, is a mediator of this effect. There is presumably some 'triggering' function of a relatively low dose of radiation for some mechanisms of infectious genetics which might be involved in inducing this phenomenon.

In order to confirm our hypothesis, an attempt was made to reveal the onset moment of the resistance depression. Thus, it would be possible to estimate the number of generations between the 'hit' of irradiation and the consequences on the characteristic of the bacteria – their resistance to tetracyclines.

We used 3 conventional healthy piglets (age 7 weeks; weight 9.5, 9.9 and 11.0 kg respectively), which were irradiated by the dose of 600 r, $\simeq LD_{50/30}$. (For the conditions of care, feeding and irradiation see the previous contribution). The piglets, which had been put on a diet without CTC immediately after they had been delivered to the institute, were sampled 8 times and their faecal coliforms tested on the resistance during the pre-irradiation period of 18 days. After irradiation the samples of faeces were taken 10 times at 3 h intervals (from 3–30 h)

[1] I. KUNSTÝŘ, I. MIKULA, A. SOKOL and V. STAVÁREK, Strahlentherapie, in press.
[2] I. PEČEVSKY and B. MILETIČ, Nature *210*, 325 (1966).

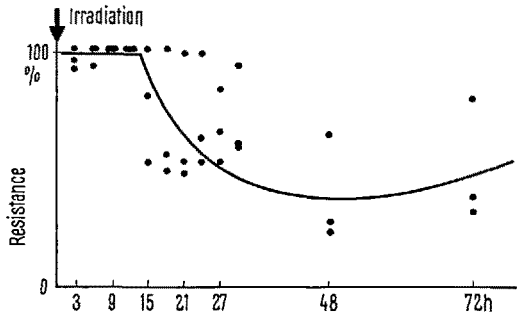

Fig. 1. The level of chlortetracycline-induced resistance to tetracyclines of faecal coliforms of conventional piglets before and after 600 r ($\simeq LD_{50/30}$) of total-body X-irradiation in days.

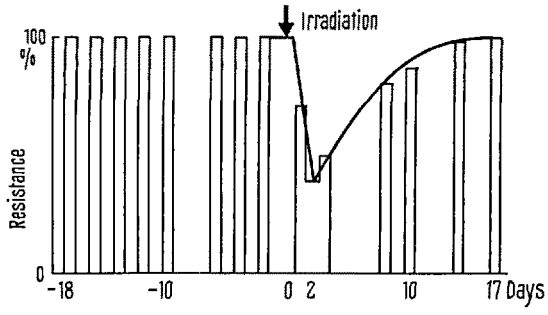

Fig. 2. The level of chlortetracycline-induced resistance to tetracyclines of faecal coliforms of 3 conventional piglets after 600 r total-body X-irradiation in hours.

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.

I. Kunstýr
and P. Otoupal

Military Veterinary Research Institute, Praha 5 (Czechoslovakia), 5 January 1968.

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

[1] J. B. Sumner, N. Gralén and I. B. Eriksson-Quensel, J. biol. Chem. *125*, 37 (1938).
[2] F. J. Reithel and J. E. Robbins, Archs Biochem. Biophys. *120*, 158 (1967).
[3] G. Gorin and C. C. Chin, Anal. Biochem. *17*, 49 (1966).
[4] G. Gorin and C. C. Chin, Fedn Proc. Fedn Am. Socs exp. Biol. *26*, 605 (1967).
[5] G. Mamiya and G. Gorin, Biochim. biophys. Acta *105*, 382 (1965).
[6] C. C. Chin and G. Gorin, Anal. Biochem. *17*, 60 (1966).