$\gamma$-radiation administered under $4\pi$ geometry in a 2400 curie radiation source[7] were used in this study. This dose resulted in 75% mortality within 20 days. From various experiments 12 animals which had served as saline controls (Group A) and 16 guinea-pigs which had been treated with cell-free mouse of guinea-pig spleen extracts (Group B) were available. Maintenance and observation of the animal took place as previously described[6].

*Results.* The following observations were made:

(a) Mortality. During the second post-irradiation year, 5 out of 12 saline-treated animals, or 41.7%, and 6 out of 16 spleen extract treated animals, or 37.5%, died. In agreement with the almost equal mortality in both groups, Table, column 2 indicates no obvious difference in death distribution. The mean survival times were 590 and 565 days, respectively. Statistical analysis by means of Student's 't' test showed that the difference in mean survival time between groups A and B was insignificant ($p > 0.60$).

Death distribution and weight changes of guinea pigs dying during the second post-irradiation year

(A) Saline controls

| Animal No. | Post-irradiation day of death | % Weight changes |
|---|---|---|
| 1346-3 | 512 | − 8.6 |
| 1346-5 | 541 | + 5.3 |
| 1346-A4 | 588 | + 20.0 |
| 1346-7 | 603 | − 12.9 |
| 1332-10 | 708 | − 2.5 |
| Average | 590 | + 1.3 |

(B) Spleen treated

| Animal No. | Post-irradiation day of death | % Weight changes |
|---|---|---|
| 1347-3 | 407 | + 99.1 |
| 1347-4 | 542 | + 79.9 |
| 1333-4 | 556 | + 94.8 |
| 1347-1 | 568 | +109.9 |
| 1333-5 | 606 | + 4.8 |
| 1348-7 | 709 | + 15.9 |
| Average | 565 | + 67.4 |

(b) Body Weight Changes. At death the body weight of the animals was established. Pertinent data are listed in column 3 of the Table which indicates that the saline-treated animals showed instances of loss of body weight as compared to the starting weight, resulting in an average weight corresponding to that of zero day of the experiment. In contrast to this, the spleen extract treated animals all showed weight gain, averaging 67% above the starting weight. Student's 't' test revealed that the difference between groups A and B was highly significant ($p < 0.02$).

(c) Autopsy Findings. The macroscopic observations made at autopsy did not indicate a specific cause of death and showed in all of the animals dying before the 600th post-irradiation day only a more or less pronounced pneumonia. In addition, a complete absence of fat depots in the saline-treated animals was noted.

*Discussion.* Death distribution and body weight changes of irradiated saline control and spleen extract treated guinea-pigs clearly indicate that the latter group of animals did not succumb eventually from an anaphylactic reaction causing severe emaciation of the animals, the so-called 'secondary disease'. On the contrary, the notable weight gain of the spleen extract treated animals observed during the acute phase of the radiation syndrome, which paralleled the reduced mortality during the first 20 days after irradiation, remained in evidence until death.

In the absence of adequate data on guinea-pig gerontology, the question whether the animals died during the second post-irradiation year due to natural or radiation-induced premature ageing must be left undecided at this time.

The importance of the present investigations for a possible clinical application of spleen extracts consists in the fact that even crude cell-free spleen extracts can be administered to guinea-pigs, a species well known for its susceptibility to allergic manifestations, without producing deleterious late effects within a period of time comparable to approximately one half of the maximum life span of this species.

*Zusammenfassung.* Todesalter und -gewicht von mit Milzextrakt behandelten Meerschweinchen und von Kontrolltieren im zweiten Jahr nach $\gamma$-Bestrahlung mit einer 75% letalen Dosis werden verglichen und das Fehlen von schädlichen Spätwirkungen der Milzextraktherapie aufgezeigt.

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## Antagonism of Chlorpromazine by $\beta$-Melanocyte Stimulating Hormone ($\beta$-MSH)

Previous communications from these laboratories have described the actions of $\beta$-melanocyte stimulating hormone $\beta$-MSH[1,2] and of chlorpromazine[3], on spinal reflexes of cat spinal cord. It was found that $\beta$-MSH potentiates spinal reflexes, whereas chlorpromazine depresses them. In an extension of the studies on chlorpromazine[4], it was found that this drug is capable of depressing the positive intermediary potential of cat spinal cord. The positive intermediary potential was initially described by GASSER and GRAHAM[5] as a negative potential associated with increased central excitatory state. The conclusions of GASSER and GRAHAM were later verified by LLOYD and MCINTYRE[6] who used somewhat modified techniques. Thus, the observation that chlorpromazine depresses the

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positive intermediary potential may be of importance, since it could conceivably explain some actions of this drug, particularly those on the reticular formation described by DEMAAR et al.[7]. In view of these observations, an investigation was undertaken to determine whether β-MSH can antagonize the action of chlorpromazine on the positive intermediary potential of cat spinal cord.

*Methods and Materials.* Seven decerebrate cats were used in this study. Three of these had spinal transections at $L_1$. The techniques used to study the positive intermediary potential were modifications of those of LLOYD and MCINTYRE[6]. A complete description is being presented elsewhere[8]. Briefly, it consists in stimulating a spinal rootlet from the last lumbar segment and electronically displaying the activity so evoked in an immediately adjacent dorsal spinal rootlet. Stimuli used in these experiments were maximal, and delivered at a frequency of 0.5 cps (typical settings of the stimulator were 0.2 msec duration and 0.05 V amplitude). Once stimulation was started, it was continued for the duration of the experiment. Decerebration was performed under ether anesthesia which was discontinued at least 1 h prior to the initiation of the experiment. Then the experiment was started only after the positive intermediary potentials had been seen to remain constant for at least 30 min[9].

*Results.* Similar results were obtained in both decerebrate and decerebrate-spinal cats. The administration of chlorpromazine in doses of 4 to 10 mg/kg resulted in inhibition of the positive intermediary potential. The subsequent administration of β-MSH in doses of 0.14 to 0.25 mg/kg resulted in restoration of the positive intermediary potential towards normal. The time course of this effect followed that described previously regarding modifications of evoked monosynaptic spinal potentials appeared approximately 4 min after injection, reaching a maximum ca. 20 min thereafter, and lasting for more than 30 min. There were no changes in blood pressure or respiration that could be associated with the alterations in positive intermediary potentials.

*Conclusions.* β-MSH is capable of antagonizing the actions of chlorpromazine probably by acting directly on the cat spinal cord. The observations reported here would be in agreement with the hypothesis that chlorpromazine acts to lower the central excitatory state by modifying the activity of β-MSH in the nervous system[10].

*Résumé.* Une préparation hautement purifiée de l'hormone mélanophorétique β-MSH inhibe les effets de la chlorpromazine au niveau de la moelle spinale.

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## The Potassium Concentration of Frog Ringer's Solution[1]

Ringer's solution for bathing organs and tissues of the frog *in vitro* is generally made to contain a potassium concentration of 2.5 mEq/l. This concentration is probably based on the average concentration of potassium in frog plasma given by FENN[2] in 1936. However, the potassium concentration of frog plasma varies with the species of frog as well as with a number of other variables. In *R. areolata*, *R. catesbeiana*, and *R. esculenta*, for example, the potassium concentration of the plasma is, respectively, 5.8, 4.8, and 5.1 mEq/l[3]. In contrast, recent determinations of the potassium content of plasma in *Rana pipiens* gave an average value of 1.7 ± 0.2 (S.D.) mEq/l based on 30 pooled samples from 90 frogs[4].

It is well known that individual intact leg muscles of the frog lose potassium when they are placed in oxygenated Ringer's solution which contains no potassium. Conversely, muscles placed in Ringer's solution which contains up to 6–10 mEq K/l, gain potassium. It follows that there is a concentration of potassium between these two extremes at which the average muscle neither gains nor loses potassium. This fact is of great importance when it is desired to study the flux of potassium in both directions across the muscle cell membrane under conditions of steady state.

From data in a previous paper[5], the steady state potassium concentration of the Ringer's solution can be interpolated to be 3.4 mEq/l for paired tibialis anticus longus and iliofibularis muscles of *R. pipiens* (see Figure 2 of ref. [5]). These were muscles of frogs kept at 5°C during the months of October and November. The average weight of each pair of muscles was 175 mg. After dissection, they were placed in Ringer's solution which contained, in m*M*/l, NaCl, 109.5; KCl, 2.0; $MgCl_2$, 1.0; $CaCl_2$, 1.26; $NaH_2PO_4$, 0.62; $Na_2HPO_4$, 3.96, and had a pH of 7.4. After 18 h at 5°C, the muscles were transferred to fresh oxygenated Ringer's solution containing 5 m*M* sodium lactate and varying concentrations of potassium and incubated at 20°C for 6 h. Lactate itself reduces the net loss of potassium from frog muscle in Ringer's solution containing 2.0 mEq K/l[5] so the steady state concentration of potassium without lactate would undoubtedly be higher than 3.4 mEq/l for these muscles.

However, potassium concentrations in the range that provides a steady state for potassium in paired tibialis anticus and iliofibularis muscles cause a net gain of potassium in the sartorius muscle of *R. pipiens* (in October and November) under the same *in vitro* conditions. The relationship between the net change in muscle potassium

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