TABLE I.
Summary of Analyses of Orbital Contents of Guinea Pigs.

	Group	No. animals	Water content, %	Hexosamine content, mg/100 mg tissue (dry wt)	Total dry w t orbital content
A	Intact controls	12	80.6 ± .4*	.70 ± .03	145.5 ± 6.4
B	Intact + TSH (5 days)	4	82.7 ± .8	.97 ± .05	156.9 ± 7.3
B	Intact and TSH (14 and 21 days)	8	80.5 ± 4	.90 ± 09	
C	Thyroidectomized	5	83.1 ± .2	.92 ± .07	122.9 ± 15.5
D	Thyroidectomized + TSH	8	86.9 ± .5	1.27 ± .11	109.7 ± 8.9

* S.E. $= \sqrt{\frac{\Sigma\Delta^2}{n(n-1)}}$

responsible for the extrusion of the ocular globe.

Summary. The development of experimental exophthalmos in the guinea pig is dependent on the accumulation of large quantities of intercellular ground substance and water in the connective tissue of the orbital contents. This is evidenced by the presence of considerable amounts of metachromatic material, of which hyaluronic acid is an important component, and by an increase in the hexosamine and water content of these tissues.

This is similar to the changes observed in localized myxedema in man, and suggests a common mechanism for the two conditions.

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Inhibition of Aerobic Respiration of Rat Brain by Desoxycorticosterone *in vitro*.* (17605)

E. EISENBERG, GILBERT S. GORDAN, HENRY W. ELLIOTT, AND JOHN TALBOT.
From the Division of Pharmacology and Experimental Therapeutics, and the Divisions of Medicine and Preventive Medicine, University of California Medical School, San Francisco.

Selye's observation that large amounts of steroids induce anesthesia,(1) together with the relationships thought to exist between anesthetics and the carbohydrate metabolism of brain,(2) have provided a basis for studies concerning the effect of steroids on rat brain cell respiration. It has been found that these agents are capable of inhibiting the aerobic respiration of rat brain, presumably at the dehydrogenase level.(3) Our previous studies indicate that this phenomenon also operates in the intact organism since (a) deprivation of gonadal steroids by castration leads to an increased rate of glucose oxidation by rat brain,(4) (b) tissues from castrated male rats differ from normal tissues in their response to steroids added *in vitro*,(5) and (c) a parallelism can be shown between the *in vitro* inhibiting potency and *in vivo* anesthetic activity of the steroids.(3)

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1. Selye, H., *Endocrinology*, 1942, v30, 437.
2. Michaelis, M., and Quastel, J. H., *Biochem. J.*, 1941, v35, 518.
3. Gordan, G. S., and Elliott, H. W., *Endocrinology*, 1947, v41, 517.
4. Eisenberg, E., Gordan, G. S., and Elliott, H. W., *Science*, 1949, v109, 337.
5. Eisenberg, E., Gordan, G. S., and Elliott, H. W., *Endocrinology*, 1949, v45, 113.

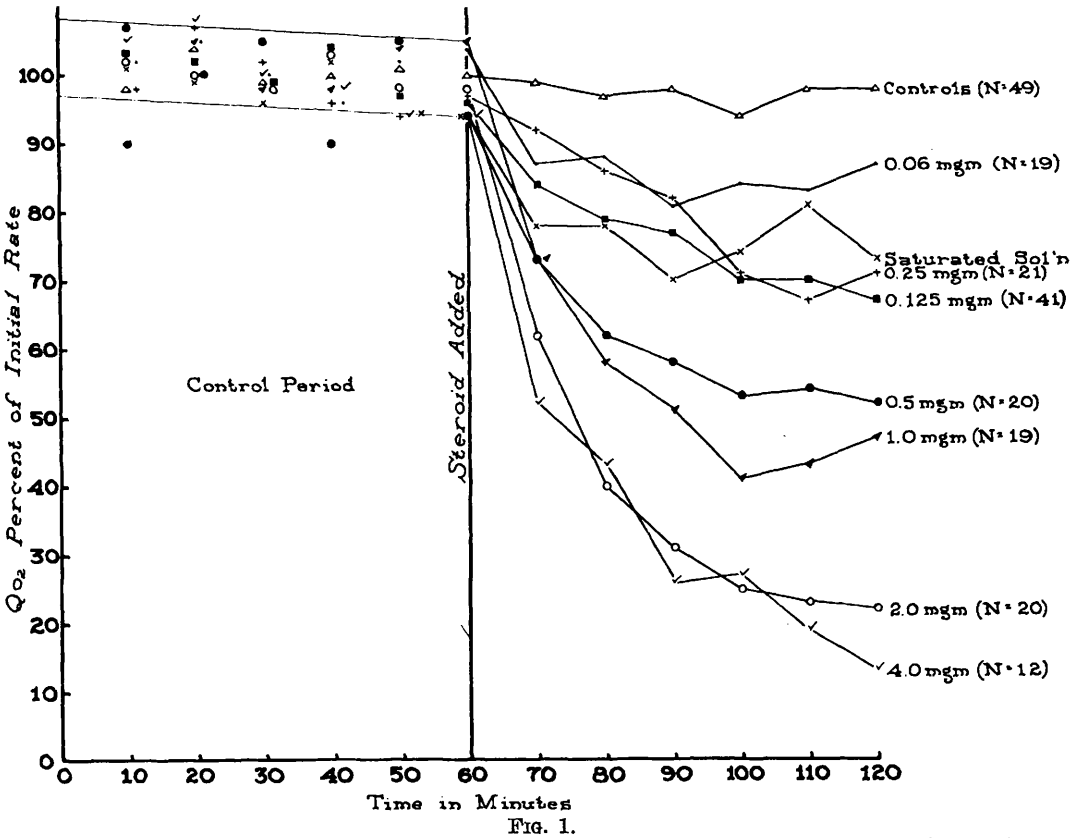

FIG. 1.

Effect of DC upon respiration of rat brain suspensions *in vitro*. (N represents number of vessels).

We have been concerned, however, by the relatively large amounts of steroid used to produce this effect upon aerobic respiration. It was our original assumption that the low aqueous solubility of the compounds would cause any excess to act simply as a reservoir, and that the solution in the flask would be saturated or supersaturated as long as any excess were present. This assumption led to the use of large amounts of suspended steroid as well as the selection of a tissue with a high lipid content dispersed as a cellular suspension to increase surface area. To study this problem further, we have compared the effect of varying concentrations of a steroid upon the oxygen consumption of a standard rat brain cell suspension. Inasmuch as desoxycorticosterone (DC) produces more inhibition than other steroids we have studied, it was selected as a steroid suitable for comparison of concentration-action relationships. Comparisons were also made using similar amounts of a water-soluble conjugate, desoxycorticosterone glucoside (DCG).†

Method. Cerebral hemispheres obtained from Slonaker-Wistar rats were made into a 10% suspension in modified glucose Ringer phosphate buffer using the Potter all-glass homogenizer. Oxygen uptake was determined at 10-minute intervals using conventional manometric technic. DC, previously ground to a fine powder and suspended in 0.2 ml of distilled water, was added from a side arm after a 60-minute control period. Readings were then made for an additional 60 min. Each flask contained 1 ml (100 mg) of brain suspension in a total final volume of 2 ml. In other experiments, aqueous solutions of DCG were similarly added.

† The steroids used in this study were generously furnished by Doctor E. Oppenheimer, Ciba Pharmaceutical Products, Inc., Summit, N.J.

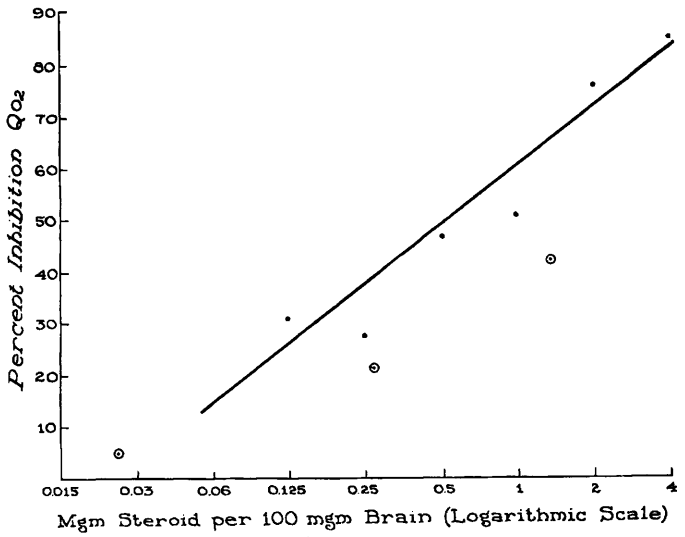

FIG. 2.

Relation between amount of desoxycorticosterone and per cent inhibition of respiration. (Solid points represent DC, circled points, DCG.)

Experimental Data for DC are plotted in Fig. 1. It will be observed that increasing amounts of steroid produce increasing degrees of inhibition. Fig. 1 also demonstrates that increasing amounts of steroid take increasingly longer periods of time to attain a maximum effect. Maximal effect, once obtained, is constant with no increase or release of this inhibition of respiration even when the experimental period is extended to 2 hours. The concentration-effect relationship seen in Fig. 1 is reasonably linear when the logarithm of the amount of steroid is plotted against the per cent inhibition (Fig. 2). Analysis of variance for the data shows no evidence of deviation from linearity. The per cent inhibition for each amount of steroid was calculated by comparing the QO_2 one hour after addition of the steroid with the average QO_2 for the 60-minute control period. This figure was corrected by subtracting the 2% drop in QO_2 observed in control vessels over the same period of time. DCG likewise shows increasing effect with larger amounts in the range studied. These points are shown in Fig. 2 as the amount of DC added (calculated from the relative molecular weights of the free steroid and the glucoside conjugate). The glucoside appears to be somewhat less potent, but this difference is difficult to assess because of a drop in QO_2 in the control flasks of this experiment.

Examination of Fig. 2 demonstrates that 0.2 ml of a saturated solution of DC produces an effect biologically comparable to that produced by 0.24 mg of the steroid (indicating a solubility of 3.6×10^{-3} M). The solubility of DC has been reported as 3.6×10^{-4} M.(6) The discrepancy between these solubilities is probably accounted for by the difference in the temperatures at which they were obtained, the larger figure being determined at 37.2°C and the smaller at 18°C.

Discussion. These data indicate that our previous hypothesis of the large amounts of steroid acting simply as a reservoir is no longer tenable. If that supposition had been correct, there should have been little difference in effect from the varying amounts used. In addition, the water-soluble conjugate produced a degree of inhibition similar to that found with the equimolar amount of unesterified steroid and in a shorter period of time. Increasing amounts of the conjugate likewise produced increasing effects.

It also appeared that the larger amounts of steroid require a longer period of time to

6. Miescher, K., Fischer, W. H., and Meystre, C., *Helv. Chim. Acta*, 1942, v25, 40.

affect the tissue, but eventually do so, leaving no reservoir of steroid in the liquid phase. To test the validity of this conclusion, the preparations resulting from 60-minute incubation of the brain preparation with 1, 2, and 4 mg of DC at 37.2°C were filtered and the filtrates examined for steroid by Zimmerman reaction and for effect on respiration of other brain suspensions. No steroid could be detected in the filtrate by either method. The amount of DC necessary to produce minimal significant inhition of aerobic respiration *in vitro* was 60 μg, a greater concentration than that which has been shown to affect other systems. For example, Bartlett and Mackay(7) have found that 30 μg of DC reduce the amount of glycogen formed when skeletal muscle is incubated with glucose.

Summary. (1) The concentration-action relationships of DC and DCG upon aerobic respiration of rat brain cell suspensions indicate:

(a) Increasing amounts of DC and DCG produce increasing amounts of inhibition This relationship is reasonably linear plotting the logarithm of the amount of DC against per cent of inhibition produced.

(b) With increasing amounts of DC a longer period of time is required to attain maximal inhibition while a comparable effect is more rapidly attained with equimolar amounts of the water-soluble conjugate, DCG. The effect, once attained, remains constant without further increase or release of the inhibition.

(c) By this bio-assay the water-solubility of DC at 37.2°C is approximately 3.6×10^{-3} Molar.

(2) These data suggest that, under the conditions used, the relatively large amounts of steroid necessary to produce inhibition of aerobic respiration *in vitro* entirely penetrate or are adsorbed by the affected system. These amounts exceed the concentrations of steroid which will affect other systems *in vitro* or that occur in living organisms. This does not necessarily exclude the possibility that steroidal inhibition of aerobic respiration may be of significance in the intact organism.

7. Bartlett, G. R., and Mackay, E. M., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 493.

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Specific Localization of Anti-Rat-Lung Serum in the Lung.* (17606)

DAVID PRESSMAN† AND HERMAN N. EISEN. (Introduced by Frank Maltaner)
From the Sloan-Kettering Institute for Cancer Research, New York City.

Up to the present time, the only direct evidence that antibodies prepared against an animal's tissue actually localize in the homologous tissue has been the demonstration, with the use of radioactive tracers, that antibodies prepared in rabbits against rat kidney or mouse kidney do localize in the kidneys of rats or mice.(1-5) In order to observe whether tissues other than kidney can concentrate antibodies from tissue-homologous antisera, or whether kidney is unique in this property, we have investigated antisera against several other rat tissues.

We now have evidence, which we are reporting here, that antibodies prepared against rat lung localize in the lung. Experiments were also carried out with antisera pre-

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† Senior Fellow, in Cancer Research, American Cancer Society Fellowship recommended by the Committee on Growth of the National Research Council.

1. Pressman, D., Eisen, H. N., and Fitzgerald, P. J., *J. Immunol.*, in press.

2. Pressman, D., and Keighley, G., *J. Immunol.*, 1948, v59, 144.

3. Pressman, D., *Cancer*, 1949, v3, 697.

4. Pressman, D., and Eisen, H. N., *J. Immunol.*, in press.

5. Pressman, D., *J. Immunol.*, 1949, v63, 375.