References and Notes

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5. True homozygotes of *Nicotiana tobacum* L. are herein referred to as Ky Iso lines.
6. Data supplied by the research department of the Brown and Williamson Tobacco Corp., Louisville, Ky.
7. Data supplied by Dr. Eugene Glock, department of research and development, American Tobacco Co., Richmond, Va.
8. This study was in connection with a project of the Kentucky Agricultural Experiment Station, and is published with approval of the director.

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Stress Modification of Drug Response

Abstract. *Rats stressed by unilateral hind-leg ligation show a significantly decreased pharmacological response to the effects of hexobarbital, meprobamate, and pentobarbital. This effect is dependent upon an intact pituitary-adrenal axis, since it is absent in both hypophysectomized and adrenalectomized animals. It can be simulated by intravenous administration of adrenocorticotrophic hormone or corticosterone. The effect of stress on drug response is not noted with barbital, a compound which is not metabolized; this effect is inhibited by treatment of the animal with a drug metabolism inhibitor such as SKF 525-A (diethylaminoethyl 2,2-diphenylpentanoate). Compounds which can stimulate adrenocorticotrophic hormone secretion or act directly on the adrenals to produce corticosteroids should thus be able to stimulate their own metabolism or that of other drugs.*

Physiological stress, whether initiated by physical insult such as immobilization, ligation, or radical surgery or by chemical stressors such as formalin, reserpine, chlorpromazine, or histamine (*1*) leads to activation of the pituitary-adrenal axis as evidenced by an increase of adrenocorticotropic hormone (ACTH) in the blood, a decrease of ascorbic acid in the adrenals, and, in the rat, an increase of corticosterone in the blood (*2*). While a host of clinically useful drugs act as chemical stressors, the effect of the stress response on drug action has not been investigated. Although adrenalectomized animals show a marked increase in duration of action of certain drugs, presumably because of decreased drug metabolism (*3*), little is known of the significance of the pituitary-adrenal system as a physiological mechanism regulating the duration of drug response. Intensification of the normal functioning of this system by a stress situation has been chosen as the means of investigating its interaction with drug responses.

Male Holtzman rats were stressed by unilateral hind-leg ligation (rubber band around triceps muscle at distal end of the femur) for 2.5 hours at which time depletion of ascorbic acid in the adrenals was at a maximum. At such a time, after chemical stressors, blood corticosterone in the rat has been reported (*2*) to rise to a peak. Groups of control and stressed animals were administered hexobarbital (100 mg/kg), meprobamate (300 mg/kg), pentobarbital (35 mg/kg), or barbital (250 mg/kg) by intraperitoneal injection. Barbiturate and meprobamate "sleep times" were measured as the criterion of drug response. Sleep time was taken as the period of loss of the righting reflex. With the exception of barbital, a highly significant decrease in the duration of action of all drugs studied was seen (Table 1).

With the sleep time associated with hexobarbital as the criterion of drug response, adrenalectomized and hypophysectomized rats were subjected to the stress situation. No effect was noted ($p > .5$) on the duration of drug action.

On the other hand, treatment of intact rats by intravenous injection of ACTH (100 mU per animal) or corticosterone (50 μg per animal) before hexobarbital treatment simulated the stress condition in that the duration of action of hexobarbital was significantly ($p < .0005$) decreased. Controls received injections of solvent, in equal volume, intravenously prior to injection of hexobarbital. Similarly, treatment of animals with 50 mg/kg of SKF 525-A (diethylaminoethyl 2,2-diphenylpentanoate) completely blocked ($p > .4$) the effect of the stress condition on the duration of action of hexobarbital. That this compound inhibits drug metabolism is well known (*4*).

These data suggest a physiological mechanism for regulating the duration of drug response in the intact animal. Results obtained with barbital and with SKF 525-A suggest that the stress effect on the duration of drug action is mediated through increased drug metabolism.

In order to examine this premise further, rats were rendered functionally hypophysectomized by a 4-day treatment with morphine (*5*), and their ability to respond to the stress situation as well as to exogenous ACTH was determined. This sequence is of especial interest since prolonged morphine treatment is also known to depress drug metabolism (*6*). Hexobarbital sleep time was again used as a criterion of drug response. There are no significant differences in the duration of action of hexobarbital between the "morphine control" group and the "morphine stress" group, whereas the morphine-treated animal shows the characteristic response to exogenous ACTH. This would be expected since adrenal responsiveness is not altered (*5*) in the morphine treated animal, but the pituitary is functionally ablated. The action of morphine in depressing drug metabolism in these experiments is suggested by the highly significant difference noted when comparing hexobarbital sleep times in animals treated only with saline (controls) and those treated with morphine (Table 2). Thus, the pituitary-adrenal axis must be intact to produce the characteristic decrease in drug response seen in the stressed animal, whereas the ultimate mediator of the response appears to be the adrenal cortex.

Smith *et al.* (*2*) have recently shown that the characteristic ascorbic-acid depletion of the adrenals and the elevation of blood corticosterone indicative of the stress response are present in the rat whose adrenal medulla has been

Table 1. Stress effects on duration of drug response. The drugs were administered after 2.5 hours of hind-leg ligation. The figures in parentheses are the number of rats in the group tested.

Adrenal ascorbic acid (mg% ± S.E.)	Duration of drug response (minutes ± S.E.)			
	Hexobarbital	Meprobamate	Pentobarbital	Barbital
		Controls		
787 ± 43 (5)	24 ± 1.1 (10)	129 ± 3.7 (7)	73 ± 2.7 (7)	221 ± 18 (8)
		Stressed		
490 ± 26 (5)	17 ± 0.95 (8)	102 ± 1.8 (7)	50 ± 1.8 (8)	266 ± 22 (8)
$p < .0005$	$p < .0005$	$p < .0005$	$p < .0005$	$p > .05$

Table 2. Inhibition of stress effect (measured by "sleep time") on drug response by morphine treatment. Rats (seven in each group) were given 20 mg of morphine per kg of body weight for 4 days; 24 hours after the last morphine injection, hexobarbital (100 mg per kg) was administered intraperitoneally.

Group	Treatment	Hexobarbital sleep time (minutes ± S.E.)*
A	Saline (1 ml per kg)	25.7 ± 1.4
B	Morphine (morphine control)	47.8 ± 1.4
C	Morphine + 2.5 hr hind-leg ligation	45.2 ± 2.2
D	Morphine + ACTH (100 mU)†	31.6 ± 1.8

* Significance: A compared to B or C, $p < .0005$; B compared to C, $p > .15$; B or C compared to D, $p < .0005$. † Milliunits intravenously.

removed. Data showing a direct action of intravenously administered physiological doses of corticosterone indicate that the suggested alteration of drug metabolism or disposition or both is promoted in the stressed rat by corticosteroids.

The implications of these findings are that pituitary-adrenal activity exerts a regulating influence on drug responses and that drug-induced stimulation or depression (morphine) of this system (characteristically at the level of the pituitary or hypothalamus) can be expected to decrease or increase the duration of drug response. Experiments with barbital, SKF 525-A, and morphine suggest that the mechanism of the response is related to stimulation of drug metabolism in the stressed animal (*7*).

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Ribosomal RNA in the Developing Chick Embryo

Abstract. *Developing chick embryos from the stage of primitive-streak formation make ribosomal (28S and 16S) RNA. The size and composition of the RNA appears to be constant throughout the first 7 days of development.*

Recent advances in molecular genetics and biochemistry have outlined the major steps in the transcription of genetic information into specific protein molecules (*1*). The active unit of protein synthesis in many different types of cells is the ribonucleoprotein particle, the ribosome, or as has recently been described, groups of ribosomes called polyribosomes (*2*). The RNA molecule (messenger RNA) which serves the function of information transport is attached to the ribosomes, but is distinguishable from ribosomal RNA on the basis of size and base composition (*1*, *3*).

It can be anticipated that these new ideas and techniques dealing with the control of protein synthesis will be useful to the embryologist, since differentiation is accompanied by changing patterns of protein synthesis in cells all of which presumably contain the same genetic potential.

As a preliminary to the study of messenger RNA in the developing chick embryo, an examination of ribosomal RNA from embryos of different ages was carried out. The size and overall composition of ribosomal RNA is constant during embryogenesis and ribosomal RNA is synthesized from the earliest embryonic stages.

Newly synthesized RNA of embryonic cells was labeled with radioisotope by two different procedures. (i) The embryo was removed from the egg and pressed through a wire screen (80 mesh). The resulting single cells and cell aggregates were collected in warmed (37°C) Eagle's medium (*4*) containing 5 percent calf serum (5 ml/embryo), and were kept in suspension by a rotating magnetic stirring bar. Radioisotope (P^{32} carrier-free orthophosphate or uridine-2-C^{14}) was either in the medium at the time of disaggregation of the embryo, or was added shortly afterward. In experiments with P^{32}, Eagle's medium containing dialyzed serum was used, and the radioactive phosphate was substituted for the usual phosphate. At the conclusion of the labeling period, the cells were centrifuged (5 min at 600*g*), and washed twice in Earle's saline solution. (ii) Radioisotope was placed directly in the upper portion of the yolk sac. The embryo was subsequently removed from the egg and disaggregated and the cells were washed as described above.

The RNA was extracted either immediately after labeling or after storage of the cells at −70°C (*5*). The base ratios of RNA represent distribution of P^{32}-labeled nucleotide in alkaline hydrolysates (*5*). The DNA was isolated as described by Marmur (*6*), and base ratios were determined after acid hydrolysis (*7*).

Sucrose-gradient analysis (*8*) of the RNA from the chick embryo revealed two larger components (28*S* and 16*S*), and a smaller (4*S*) component (see OD_{260} in Fig. 1). This size distribution of RNA has been observed in many different kinds of cells, and the larger components have been shown to be derived from ribosomes. These will, therefore, be referred to as ribosomal RNA. The apparent large amount of 4*S* RNA in Fig. 1 was not always ob-

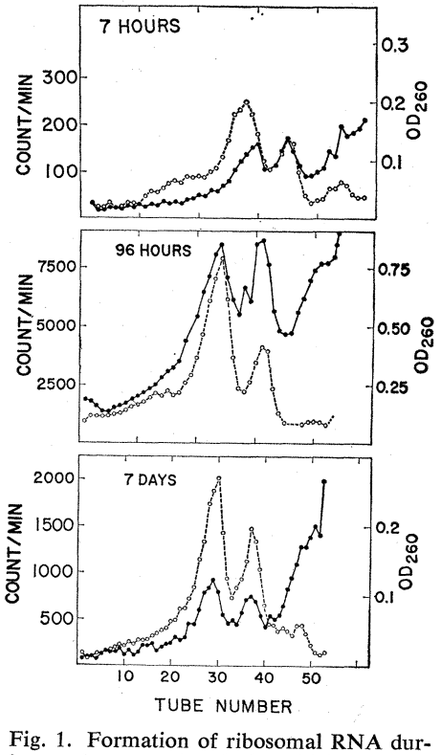

Fig. 1. Formation of ribosomal RNA during embryogenesis of the chick embryo. 200 μc P^{32} were placed into the yolk sac and RNA was extracted (*4*) from several embryos at each age along with extract from two 7-day chick embryos which served as the reference for the OD measurements. The 7-hour sample was derived from about ten embryos, 3-hours old labeled for 4 hours; the other samples were labeled for the 16 hours concluding at 96 hours and 7 days.