Reports

Approximation to a Gravity-Free Situation for the Human Organism Achievable at Moderate Expense

So far as their effects on the human organism are concerned, the chief peculiarities of weightlessness consist in (i) the cessation of unidirectional stimulation of the vestibular system, together with the sequelae accruing therefrom through reactions of the autonomic and central nervous systems, and (ii) the letting up of the hydrostatic drag on the circulatory system, especially that associated with the erect posture of man. Both of these peculiarities can be approximated to a considerable degree by a combination of relatively simple devices. The use of these would enable data on the effects of this pseudo-weightlessness, maintained for several hours at least, to be obtained long before the still exceedingly costly direct tests of subjecting human beings to prolonged free fall can be carried out by Western scientists.

By far the greater portion of the hydrostatic drag is absent in human beings whose body axis is in a horizontal position, as it is when they are recumbent. Most of the remainder can be evened out and in effect nullified by subjecting them to a moderate spinning motion about their horizontal axis, through the automatic rotation of a cylinder within which they are held. At the same time, little sense of the pressure caused by their body weight would remain if the body, including the limbs, were encased in a skin-tight envelope, and held immersed in a brine having the same specific gravity as the average for the body itself.

Considerable freedom of movement can be allowed for the limbs. The head can be encased in a transparent helmet that is serviced for respiration and oral communication. It is to be held with its axis in alignment with the body axis. That is, the head is not permitted tilting movements that would set its axis at an angle to that of the body; however, it is left free to carry out any desired voluntary movements of rotation on its axis. A field of view, imitative of furnishings and, for example, of a window showing a skyscape, would be arranged that remained in a fixed position with reference to the subject. Thus the field of view would spin together with the subject himself, and the subject would lack the visual stimuli associated with an imposed rotary movement.

The subject, after having been fastened within the cylinder, would at first be at rest but by insensible degrees would be subjected to a rotary movement about his horizontal axis, at a speed that increased until it attained the psychophysiological optimum for disengaging his vestibular apparatus from an effective pull by gravity in any given direction. Thereafter the motion is to be kept smooth and steady. Preliminary experiments have shown that under such circumstances, so long as the subject's head remains with its original relation to the body axis, he soon becomes quite unaware of the rotary movement as such. This is because the fluid in his semicircular canals has come to rest, in relation to their walls (except for any voluntary axial turning movements, which then give rise only to the effects usual for them), and because he has no notification of the imposed rotation through vision and very little through skin or internal bodily pressures.

It is likely that a suitable speed of rotation could be found which was too fast, in relation to the sensitivity of that part of the vestibular apparatus which detects translational (linear) acceleration or gravity in any one direction, to allow such stimuli to accumulate to an appreciable degree. That is, not so rapid a periodicity should be required to transcend "flicker" in the case of the sensation of linear acceleration here in question as in the case of optical flashes. For psychological and physiological purposes, a condition approximating that of weightlessness or free fall would thereby have been achieved. Essentially the same mechanism has long been used for nullifying gravity in studies on plants, but the speed of rotation for this apparatus, called a clinostat, can be much slower because of the much slower reactivity of plant tissues.

Among other questions that would thereby be opened for investigation are those concerned with the effects, on free-fall tolerance, of individual differences (as between persons of differing tendencies to become giddy or motion-sick) and of the effects of differing physiological conditions and of diverse drugs (such as those used against motion sickness). The relatively small cost of the apparatus required for such experiments, and the relatively short time required for its construction, recommend it for pilot studies on the effects of fairly prolonged weightlessness (*1*).

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Note

1. This report is based upon a paper read at Symposium on Possible Uses of Earth Satellites for Life-Sciences Experiments, Washington, D.C., 17 May 1958; Contribution No. 659 of Zoology Department, Indiana University.

21 May 1958

Role of Somatotropin in Mammogenesis and Lactogenesis in C3H/He CRGL Mice

In mammary gland growth and differentiation in rodents, mammotropin with ovarian steroids induces lobulo-alveolar development, and if this is followed by treatment with mammotropin and adrenocortical steroids, lactogenesis will result (*1*). Somatotropin (hypophysial growth hormone) synergizes in both these cases to enhance the action of the hormonal combinations (*2, 3*); however, somatotropin has not been reported to act either as a lobulo-alveolar mammogen or as a lactogen in the absence of mammotropin.

Recently it was shown that a purified somatotropin preparation (containing 0.5 to 2 percent mammotropin) acts alone as a duct mammogen in hypophysectomized rats (*1*). Ferguson (*4*) observed that this hormone by itself had no direct mammogenic action in hypophysectomized C3H mice but that the formation of terminal buds and some alveoli was induced by treatment with somatotropin, estradiol, and progesterone. The present report is concerned with the role of somatotropin in mammogenesis and lactogenesis in C3H/He CRGL mice. These experiments are part of a larger study dealing with the endocrine con-

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

trol of mammary growth and function in this strain of mouse (*5*).

Pituitaries, ovaries, and adrenals were removed (triply operated) from 3-month-old virgin C3H/He CRGL females. The animals were then treated for 15 to 20 days with various combinations of ovarian and adrenocortical steroids together with somatotropin, administered in daily subcutaneous injections. The steroids were prepared as aqueous suspensions, and unless otherwise indicated, the following daily doses were used: β-estradiol, 1 μg; progesterone, 1 mg; deoxycorticosterone acetate, 0.2 mg; cortisol acetate, 125 μg. Various amounts of bovine somatotropin (15 μg to 1 mg), dissolved in water at *p*H 8.0 to 8.5, were also administered daily. At the end of each experiment, the mammary glands were removed, fixed in 10 percent formalin, stained with iron-hematoxylin, cleared, and stored in methyl salicylate. These preparations were examined under a dissecting microscope, after which representative areas were taken for histologic study.

The mammary glands of normal 3-month-old virgin females usually contain only medium and wide ducts and terminal buds of various sizes. Occasionally, a few alveoli and alveolar buds are also present. Removal of pituitaries, ovaries, and adrenals results in regression of the finer branches and terminal buds within 15 days (Fig. 1, *A*).

Two hormonal regimens, involving somatotropin, given for 20 days, were found to stimulate the duct system in triply operated mice beyond the level of development seen in 3-month-old controls: Daily administration of 0.5 mg of somatotropin, along with estradiol and deoxycorticosterone acetate, or along with estradiol, deoxycorticosterone acetate, and 62.5 μg of cortisol acetate, resulted in comparable degrees of duct stimulation (Fig. 1, *B*).

Somatotropin, along with estradiol and progesterone, was found to induce lobulo-alveolar development in triply operated mice. Treatment with estradiol, progesterone, and 15 μg of somatotropin per day for 15 days resulted in the formation of a few alveoli. When the level of somatotropin was increased to 100 μg/day, large numbers of alveoli, some organized into lobules, were found. Increase of somatotropin dosage to 1 mg/day resulted in lobulo-alveolar development similar to that of intact females at midpregnancy. This lobulo-alveolar development was at least as extensive as that obtained with estradiol, progesterone, and 1 mg of mammotropin per day (*5*).

Lactogenesis was induced in triply operated females by daily injections of estradiol, progesterone, and 1 mg of somatotropin for 15 days, followed by daily injections of cortisol acetate and 1 mg of somatotropin for 5 days. Glands from mice treated in this way were composed of lobules filled with milk (Fig. 1, *C*), and the secretion-filled alveolar lumina were partially lined with vacuolated cells (Fig. 1, *D*), such as are found in the normal lactating mammary gland. These results are similar to those obtained experimentally when mammotropin is used in place of somatotropin (*5*).

Additional experiments eliminate other possible explanations for the observed effects of somatotropin: (i) A mouse placentoma assay indicated the presence of 0.5 to 1.0 percent mammotropin in our somatotropin preparation. Neither lobulo-alveolar development nor lactogenesis was induced in triply operated mice when the somatotropin was replaced by small amounts (15 μg/day) of mammotropin, indicating that the results obtained with our somatotropin preparation could not be ascribed to the small quantity of mammotropin present as a contaminant. (ii) Serial sections of operated areas from the skulls of representative triply operated animals showed no residual pituitary tissue, thus excluding the possibility that such remnants might be secreting hormones that could synergize with the exogenous somatotropin. (iii) Mice hypophysectomized and ovariectomized 20 to 25 days before initiation of treatment showed the same responses as those treated immediately

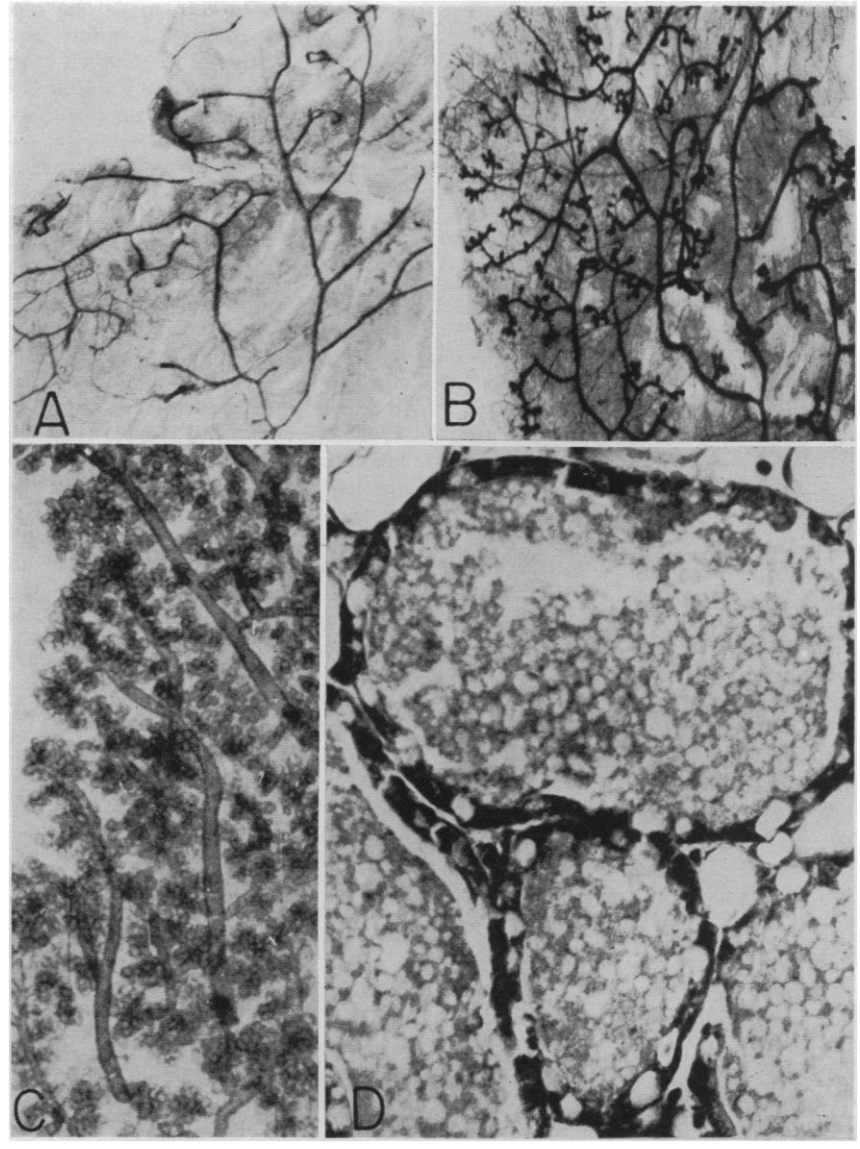

Fig. 1 (*A*) Mammary gland whole-mount from 3½-month-old female, 15 days after removal of pituitary, ovaries, and adrenals. Hematoxylin. (×7). (*B*) Mammary gland whole-mount from triply operated female treated for 20 days with estradiol, deoxycorticosterone acetate, and somatotropin. Hematoxylin. (×7). (*C*) Mammary gland whole-mount from triply operated female treated with estradiol, progesterone, and somatotropin daily for 15 days, followed by cortisol acetate and somatotropin daily for 5 days. Hematoxylin. (×15). (*D*) Section through a part of the mammary gland seen in *C*. Masson. (×400).

postoperatively. Hence, the observed effects of somatotropin are not the result of synergism with pituitary hormones that might remain in the blood for a short time after hypophysectomy.

Our observations indicate that somatotropin can synergize with appropriate ovarian or adrenocortical steroids, or both, to induce ductal development, lobulo-alveolar development, and lactogenesis in the mammary glands of triply operated C3H/He CRGL female mice. Unlike the rat (*1*), our C3H mouse is responsive to the mammogenic and lactogenic actions of somatotropin even in the absence of appreciable amounts of mammotropin (*6*).

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References and Notes

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2. ———, *J. Clin. Endocrinol. and Metabolism* 12, 45 (1952).
3. W. R. Lyons, C. H. Li, R. D. Cole, R. E. Johnson, *ibid.* 13, 836 (1953).
4. D. J. Ferguson, *Surgery* 39, 30 (1956).
5. S. Nandi, Ph.D. thesis, Univ. of California, Berkeley (1958); *Univ. Calif. (Berkeley) Publs. Zoöl.*, in press.
6. I am grateful to Professors H. A. Bern and K. B. DeOme for their suggestions; to Professor C. H. Li for the generous supply of purified bovine somatotropin (L2732D-A); to Dr. G. K. Hawkins, Schering Corporation, for the β-estradiol, progesterone, and deoxycorticosterone acetate; and to Dr. J. R. Beem of Merck, Sharp and Dohme, for the cortisol acetate. This work was supported by cancer research funds of the University of California and by grants (MOR-27 and E-11) from the American Cancer Society.

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Intrasubject Conditioning as a Function of the Intensity of the Unconditioned Stimulus

Abstract. Subjects conditioned concurrently to two different conditioned stimuli, light and tone, exhibited a significantly higher level of conditioning to the stimulus paired with a strong unconditioned stimulus than to the stimulus paired with a weak one. The findings suggest that habit strength in aversive conditioning varies with the intensity of the unconditioned stimulus.

In a number of experiments (*1*) concerned with the problem of whether habit strength (H) is a function of the intensity of the unconditioned stimulus (UCS), the conditioning performances of two different groups of subjects (S's), equated for level of drive (D) but differing with respect to reinforcement conditions, were compared. The finding of a higher level of performance on the part of S's that received a strong UCS on trials producing conditioning (that is, habit growth) as compared with S's who were given a weak UCS on such trials was interpreted to mean that habit strength (H) varies with the level of intensity (noxiousness) of the UCS.

In the present experiment (*2*) we attempted to obtain further evidence on this problem by comparing the conditioning performances of a single group of S's under two different reinforcement intensities. All S's were conditioned concurrently to two different conditioned stimuli (CS's) (light and tone). Half of the S's had the light paired with a strong UCS and the tone paired with a weak UCS. In the case of the other half of the S's these relations were reversed, the tone being paired with the strong UCS and the light with the weak UCS. Our primary interest lay in a comparison of the level of conditioning performance to the CS paired with the strong UCS with that to the CS paired with the weak UCS. On the basis of the findings of the previous experiments and the interpretation offered by them, namely, that a greater amount of H is established to a CS paired with a strong UCS than to one paired with a weak UCS, it may be predicted that a higher level of conditioning performance will be made to the CS paired with the stronger UCS.

Twenty-four men and 24 women from an introductory course in psychology served as S's in this experiment. Nine other S's were eliminated, including three who met the criterion defining a voluntary responder (*3*), two who gave conditioned responses (CR's) to initial test trial presentations of the CS, and four who adapted to the air puff. An S was considered to have adapted to the puff if the mean amplitude of the unconditioned responses (UCR's) made on the last 10 trials involving the weak puff was less than 50 percent of that on the first 10 weak puff trials.

The apparatus was the same as that used in earlier studies (*1*) except for the addition of a 1000-cycle tone CS produced by a loudspeaker driven by a Hewlett-Packard oscillator.

Instructions and preliminary trials were similar to those of the previous studies. A variable (15, 20, 25 sec) intertrial interval was used, as well as a ready signal which preceded the onset of the CS by 2, 3, or 4 seconds.

Each S received 100 conditioning trials, 50 of which involved a tone CS and 50 a light CS. The two CS's were given in a prearranged sequence within which neither of the CS's occurred more than twice in succession. For half of the S's the tone was always paired with a strong (2 lb/in.^{2}) puff and the light with a weak (0.33 lb/in.^{2}) puff, while the other half received the reverse pairing, a 0.33 lb/in.^{2} puff with the light and a 2 lb/in.^{2} puff with the tone. The CS-UCS interval was 500 msec, with the duration of the CS 550 msec and the duration of the UCS 50 msec. At the end of the experiment all S's were questioned regarding their understanding of the purpose of the experiment and cautioned not to discuss the experiment with other members of the class.

Table 1. Summary of analysis of variance of frequency of CR's for trials 1 to 20 and 61 to 80.

Source	*df*	*MS*	*F*	*P*
Between S's	47			
A B (b)	1			
Error (b)	46	14.94		
Within S's	144			
CS (A)	1	40.33	11.86	.005
Puff (B)	1	44.08	12.96	.001
Trials (C)	1	752.08	191.37	.001
A C	1	3.01	1.11	> .200
B C	1	18.76	6.90	.025
A B (b) C	1	1.34	< 1.00	
Error W	138	3.35		
$Error_1$ W	46	3.40		
$Error_2$ W	46	3.93		
$Error_3$ W	46	2.72		
Total	191			

Acquisition curves in terms of the number of anticipatory CR's given to the CS paired with the strong UCS and to the CS paired with the weak UCS are shown in Fig. 1. As may be observed, the curve for the strong puff is consistently above the curve for the weak puff, with the difference gradually increasing during the course of conditioning. A summary of an analysis of variance, involving three within-subjects factors and one between-subjects interaction (*4*, p. 279, Type VII), based upon the first and last blocks of ten conditioning trials is presented in Table 1. As may be seen from the lower portion of this table, the differential effect of the UCS variable was highly significant, the obtained F providing a P value of .001. Also of interest is the fact that the interaction between the UCS variable and blocks of conditioning trials was significant (P = .025), thus indicating that the diver-

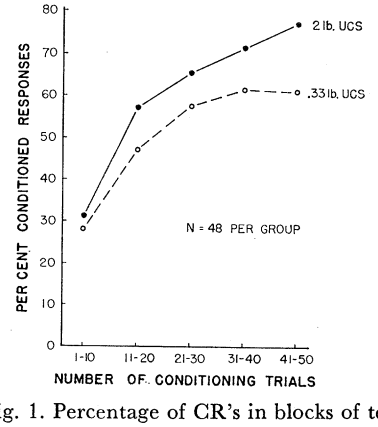

Fig. 1. Percentage of CR's in blocks of ten trials made to CS paired with 2-lb UCS and 0.33-lb UCS.