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ADVENTURES IN BIOLOGICAL ENGINEERING[1]

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MAN, together with other higher vertebrates, has developed some elegant automatic mechanisms for regulating the physical and chemical properties of his blood and body fluids. The relative constancy of one's internal environment in the face of external stress and change is characteristic of such factors as blood volume, blood sugar, hydrogen ion concentration and salt content of the body fluids. The thermostatic regulation of internal body temperature is another case in point. These factors are beautifully controlled with little or no conscious thought on our part. As Claude Bernard pointed out nearly a century ago this regulation renders the higher vertebrates free of their external environment to a degree impossible for animals not possessing these automatic mechanisms. Homeostasis of the internal environment, as Cannon, Barcroft and others have demonstrated, is one of the truly central problems of physiology.

When, for example, the environmental temperature falls a bird or mammal conserves more of its metabolic heat and maintains its internal temperature constant. A frog, on the other hand, must take on the temperature of its environment. In cold weather its metabolism and other dependent reactions are slowed until it becomes immobilized and a prisoner of the climate. Freedom thus is not just a matter of sociology and politics, but freedom of a sort has its substratum in biochemistry and physiology.

In recent years with the development of aviation man has desired to be free in an environment for which his evolutionary history could not possibly have fitted him. In high-speed airplanes he is assailed by new and formidable stresses. Living as he does at the bottom of a sea of air supplying a continuous and

[1] Sigma Xi initiation lecture given at Worcester Polytechnic Institute on June 14, 1944.

plentiful amount of oxygen he has been unprepared by evolution to store oxygen in his body as he has been able to store water and foodstuffs that come to him only at intervals. He therefore must carry his oxygen supply with him when he flies to high altitudes. He must also adjust to severe and unaccustomed accelerations and rapid changes of atmospheric pressures which, together with the peculiar emotional stresses of modern aviation, may be exacting in the extreme. Since we can not wait for evolution to prepare us to cope with this new environment it is the primary task of aviation medicine to devise means of preventing the stresses of flight from interfering too greatly with our homeostatic mechanisms.

This evening I should like to consider how some of the stresses of flying and of flight type operations may be reflected in the urinary excretion of hormones from the adrenal cortex and how the administration of certain hormones and hormone type substances may affect performance in fatiguing psychomotor tasks encountered not only in aviation but also in industry.

We shall not be concerned with problems involving the adrenal medulla. Thanks to intensive work over the past forty years the role of adrenin or epinephrin, the hormone from the adrenal medulla, is well understood. Physiologists and biochemists in recent years have only begun to understand the more complicated story of the adrenal cortex. Despite the fact that cortex and medulla are parts of the same small gland the functions of these structures are very different and the types of hormones they synthesize belong to chemically quite distinct families.

Some twenty hormones have been extracted from the adrenal cortex. They all belong to the class of substances known as steroids—four carbon ring structures with relatively simple side chains, and with molecular weights of around 300. The sexual apparatus, testes, ovaries, corpus luteum and placenta also manufacture and secrete a variety of steroids differing only in minor structural details from each other and from the hormones of the adrenal cortex. For example, slight differences only in molecular configuration differentiate the oestrogens which regulate the sexual cycle of the female from the male sex hormones of the testes and from the steroids of the adrenal cortex concerned with essential life maintaining processes such as salt and water balance and aspects of sugar storage and metabolism. The situation is not clarified by the finding that a few of the same steroids are manufactured and released both by the sexual apparatus and, to a lesser degree, by the adrenal cortex as well.

Steroids are excreted in the urine primarily in the form of 17-ketosteroids, that is as steroids with an oxygen atom attached to a particular carbon in one of the rings. There are 17 carbon atoms in the four ring steroid skeleton and the oxygen or keto radical is on the conventionally numbered 17th carbon atom. The 17-ketosteroids are thus end products of steroid hormone metabolism and, for the most part, are hormonally inactive. It is believed that the 17-ketosteroids come primarily from adrenal cortical chemical precursors rather than from precursors of the gonads because (a) in Addison's disease in which the adrenal cortex becomes non-functional there is a decline in the output of 17-ketosteroids corresponding to the severity of the disease, (b) in certain cancerous growths of the adrenal cortex there is a marked outpouring of 17-ketosteroids, (c) in ovariectomized women with healthy adrenals the output is normal and (d) in castrated males the output is 80 to 90 per cent. of normal.[2] The average output per 24 hours for healthy normal men is 7 to 27 mg and for women 5 to 16 mg. The 17-ketosteroids are extracted from the urine by organic solvents and are finally quantitatively determined with a spectrum photometer.

Available evidence indicates that hormones of the adrenal cortex play a significant role when the organism encounters stressful situations. Animals forced to severe exercise, or exposed to very low oxygen tensions or extremes of cold first display a hyperplasia of the cells of the adrenal cortex followed later by atrophy. It has been well established that adrenalectomized animals early show marked weakness and lassitude preceding collapse and death, and that they do not withstand stressful situations. The syndrome of Addison's disease in man is typically characterized by lassitude and exhaustion. Adrenalectomized animals and Addisonian patients can be maintained in a healthy state by adrenal cortex hormones.

As a result of association with a group of navy instructor pilots in the summer of 1940 I became impressed with the fatiguing character of their work. Flight instruction of 90 hours per month was very tiring for some men. As a result of discussion of this matter with my colleague, Dr. Gregory Pincus,[3] he suggested the possibility that adrenal cortical function might be related to this type of fatigue and that hormone medication might prove of value in combatting it. We accordingly decided to investigate the matter.

Our first task was to determine the output of urinary 17-ketosteroids at intervals during the day in normal

[2] For citations to these matters and to other points discussed in this lecture the reader is referred to G. Pincus and H. Hoagland, *Jour. of Aviation Med.*, S. 14: 173, 1943; and G. Pincus and H. Hoagland, *Jour. of Aviation Med.*, 15: 98, 1944. These two papers present the data of the fatigue studies described below with the exception of our as yet unpublished studies of industrial fatigue.

[3] Laboratory director of the Worcester Foundation for Experimental Biology.

healthy young men going about their daily, and not especially stressful, routine. Modifications, made in our laboratory, of standard procedures rendered it possible to make reliable 17-ketosteroid determinations on samples of urine, as small as typical one-hour collections. We accordingly made 97 determinations over a period of days on seven men working in our laboratory. While much individual variation in levels of output were encountered we were at once struck with the fact that sleep excretions were low and rose on an average of 60 per cent. in the hours immediately after waking, falling in an essentially linear fashion during the course of the day and reaching minimal levels again at night. This diurnal rhythm was a true sleep-waking rhythm since a reversal of night and day activities reversed the rhythm. It was as if the process of waking and starting the day itself produced a stress response of considerable magnitude from the adrenal cortex.

Since aviation personnel was not at first available we next proceeded to study a group of male Clark University student subjects in our own laboratory, who were subjected to the fatiguing operations of a pursuit meter. In our early series of tests we used an apparatus designed by Dr. Smith Stevens, of Harvard University, consisting of airplane type controls that moved a bar of light over prescribed pathways across a photosensitive screen. Errors were recorded automatically when the light strayed off the pathways. Continuous operation of this apparatus for two or three hours was very fatiguing and with skilled operators, *i.e.*, those having had 10 to 15 hours of practice, the score of errors increased measurably in the second halves of the tests.

Carefully timed control urine samples were collected covering at least a two hour period just before each run, and experimental samples were taken immediately after the fatiguing bout which lasted from two to six hours. Since we had observed that time of day has an effect on the relative excretion level of 17-ketosteroids we corrected each control from our standard curve to make it correspond to the time of day of the experimental sample. In this way arithmetic differences between "flight" and control samples in terms of mg ketosteroids per unit of time could be compared for a given test. We were interested to see that the fatiguing bout considerably augmented the output of 17-ketosteroids. The increased output under stress after subtracting the control values corrected for time of day to coincide with the time of the experiment we shall hereafter refer to as stress 17-ketosteroids.

Sixty-nine test runs on seven men brought out some rather striking results when we plotted each man's mean stress 17-ketosteroids against his error scores for the second halves of his test. Each individual displayed a characteristic performance level and a corresponding level of output of stress 17-ketosteroids. The men turning up the best performances showed little increase in output of 17-ketosteroids over their control values, while those showing high error scores in the second halves of the tests yielded consistently a high output of stress 17-ketosteroids. The urinary steroids were, in short, an index of scoring ability in long runs on the Stevens apparatus especially in the second halves of fatiguing bouts. The men best conserving their steroids were the best performers.

In addition we found a marked increase in urine volume excreted per unit time during the tests as compared to controls and with somewhat less regularity this augmented diuresis paralleled poorness of performance. Not only did these relationships hold between men, *i.e.*, the poorer performers excreting more 17-ketosteroids and also more urine but it held within men, *i.e.*, the individual tended to score best when his "flight" increase in urine volume or stress 17-ketosteroid level is least. For stress 17-ketosteroids as against all scores the correlation coefficient, r, was 0.723. It was 0.519 for diuresis and all scores. For scores *within* men these values were respectively $r = 0.686$ and 0.530. All four correlations were significant with P values of < 0.01. This urinary stress effect is apparently quite general since we have found it with a variety of stressful situations some of which will be described in detail later. For example, urine samples were obtained on five university students just before and just after they undertook final or doctoral examinations lasting from one to three hours. The 17-ketosteroid output and urine volume excreted per unit time was raised by the stress of the examinations as compared to the control samples.

What is the relationship between the enhanced water and steroid output? Is the increase in urine volume under stress due to enhanced formation of metabolic water as a by-product of increased muscle tone which then merely washes out more steroid material? This is evidently not the case. Pincus[4] has shown that excessive water intake with resulting marked diuresis does not increase the output of 17-ketosteroids and this finding has been confirmed by others. In fact, our evidence indicates that the enhanced hormone excretion from stress precedes, if anything, the diuresis. Presumably hormones from the adrenal cortex, some of which are known to be concerned with water and electrolyte balance, bring about the enhanced water excretion, although it is possible that both effects are by-products of some as yet unknown mechanism perhaps involving the direct

[4] G. Pincus, *Jour. Clin. Endocrinol.*, 3: 195, 1943.

action of pituitary hormones under nervous control by the hypothalamus.

The Stevens serial coordination meter originally designed for other purposes was not altogether satisfactory for our needs and accordingly Dr. Nicholas Werthessen, Mr. Murray Edinburgh and I designed and built an apparatus which better approximates certain aspects of flight. This apparatus consists of airplane stick and rudder pedal controls that operate a pointing rectangular light beam. At approximately eight feet in front of the operator an airplane model mounted on the end of a rod is caused to move in a thoroughly random and nonrepeating fashion, banking to left and right and moving up and down. In the two "engine nacelles" of the model are photoelectric cells. The operator must "fly in tight formation" with this model by simultaneously bracketting both photoelectric cells with his rectangular beam of light. He is automatically scored for his ability to do this; a counter registers each time he slips off one or both cells with his pointing light and clocks register his per cent. time on target for any chosen interval. In this way, for example, by taking five minute readings over a continuous run of an hour a curve of performance can be plotted characteristic of the individual. For practiced operators these curves show a decline characteristic of the individual during the test. A single numerical measure of fatigue can be had by dividing the mean score for the first half of a run into that for the second half. Thus, a fall of this ratio below unity for a skilled operator is a measure of his fatiguing. For most people seven to ten hours of practice are needed before the learning curve is flat although, because of "transfer of learning," professional aviators usually show flat learning curves in two to three hours. Army pilots who have used this apparatus say it is about as fatiguing as close formation flying under poor weather conditions.

Since responses to anoxia are of interest in aviation our first use of the target meter was to observe the effects of breathing mixtures of air containing reduced amounts of oxygen on scoring ability and on the excretion of 17-ketosteroids. Twenty-seven experiments were carried out on three young men in a gas chamber at the Harvard Fatigue Laboratory after they had completed a series of learning runs. Oxygen mixtures, at sea-level pressures, from 21 to 13 per cent. were used and CO_2 was absorbed by soda lime. This lower value of 13 per cent. oxygen corresponds to an altitude of about 14,000 feet in terms of oxygen saturation of the blood hemoglobin. The results were interesting in that measurable decline in performance curves were seen at oxygen levels corresponding to altitudes as low as 8,000 feet and became progressively more accentuated at lower oxygen percentages. With declining performance at decreasing oxygen tensions the stress 17-ketosteroid output rose steeply, as did the excessive output of urine. The combined stresses of low oxygen and target meter performance evidently called strongly on adrenal cortical reserves.

At about this time we were fortunate to be invited to make a study of a volunteer group of Army instructor pilots at a southern air training center. The measurement of the stress of flight was at first a source of much puzzlement but an ingenious suggestion of Dr. Pincus enabled us to measure quantitatively the effects of flight stress on 17-ketosteroid output. The method was as follows: Each pilot before putting on his flying clothes emptied his bladder and accurately recorded the time of voiding. At the end of the period of flying, on his return to the locker room, he urinated into a bottle and again recorded the time. In addition he also recorded the actual duration of time spent in the air. Thus for any flight lasting from one to four hours we obtained a urine specimen which had collected in the pilot's bladder not only during the time spent in the air but also during time on the ground just prior to the take-off and for the period after landing while the machine was being brought to the line and the pilot was checking in, returning to the locker room and changing clothes. There was considerable variation in the percentage of time a pilot was actually air-borne. Some flew only 30 per cent. of the time over which the sample collected while others were in the air as much as 95 per cent. of the time. From the data the percentage of total time that the pilot was actually air-borne could be calculated. In addition, for each of these flight samples the pilot furnished, on the same day, a separate timed control specimen of his urine. Data were thus obtained from 16 pilots for 152 flights and control samples and a plot was made of the stress 17-ketosteroids against the percentage of the time the pilot was air-borne for each flight. The results were interesting—a linear relation was obtained between per cent. increase in stress 17-ketosteroids and per cent. of time in the air such that the greater the time in the air the greater was the steroid output. The correlation was $r = 0.978 \pm < 0.01$. A repetition of this experiment carried out by us on seven Pratt & Whitney test pilots during 56 flights gave a similar result. In this series r equalled $0.922 \pm < 0.01$.

From our laboratory experiments we had noted a positive relationship between tendency to fatigue, *i.e.*, to score badly in the latter part of tests and the excretion of stress 17-ketosteroids. We accordingly asked the commanding officer to grade the sixteen pilots of his squadron who were our experimental subjects in terms of his opinion as to their fatigability putting the least fatigable man at the top of his list. The pilots were teaching flight some 90 hours per month and were well known personally to their commander from months of association. We, in turn, rated these men entirely from their urine data putting the lowest excreter of stress 17-ketosteroids at the top of the list. On matching the two rating tables we were impressed with the correspondence. Our numbers 1, 13, 14, 15 and 16 men agreed on both listings. There were many discrepancies for men in between but with, none the less, a tendency to agreement as indicated by a correlation coefficient of 0.676 with a P value of < 0.01 between our urinary index and the commander's subjective evaluation of his men's ability "to take it."

From what has been said it appears that marked variations in the abilities of men to withstand fatiguing ordeals is related to their adrenal cortical functions and it was natural to ask if the administration of suitable steroids might increase one's ability to withstand the type of measurable stress we were investigating. We have accordingly tested several steroid substances and have found one in particular, Δ^5 pregnenolone, a synthetic compound supplied to us by the Schering Corporation, that has improved and prolonged scoring ability on our pursuitmeters without having any deleterious after effects. We have compared the action of this substance to that of placebo pills that look and taste just like it but which contain no active principle. From 356 experiments on 21 healthy young men we have found, in most cases, marked improvement in target meter performance when they orally took 50 mg per day of pregnenolone.[5] In addition, comparable experiments on a group of over 100 volunteer industrial workers in two industrial war industries engaged in a variety of operations involving incentive piece work pay have shown statistically significant improvement in production rates and in waste saving when taking pregnenolone as contrasted to placebos (unpublished).

Objectively measured antifatigue effects of pregnenolone are variable from person to person. Some show no improvement and report no subjective symptoms from its use but a substantial number have reported feeling less fatigued and better able to cope with their jobs when they were taking it as contrasted to periods when they were taking placebos. That pregnenolone when given orally is absorbed and transformed in the body into a variety of hormonally active steroid substances has been demonstrated in our laboratory (unpublished). From urine analyses of aviators who took pregnenolone during actual flight tests their curve of percent time in air vs. stress 17-ketosteroids rises at only approximately half the rate of the curve when not on pregnenolone. This we believe indicates a sparing action of hormone secretion on the adrenal cortex when pregnenolone is administered. But just how or why this substance should alleviate fatigue remains for future investigations to demonstrate.

Other steroids may prove to be as efficacious in counteracting psychomotor fatigue as is pregnenolone. We obtained negative results with adrenal cortical extract and progesterone in a series of tests, but these do not rule out the possibility that these substances may counteract fatigue since the amounts we used in our preliminary tests and the number of our experiments may have been inadequate to show possible effects.

We are convinced that pregnenolone is most effective in combatting fatigue where motivation is high and where men are working under really trying conditions. Our hard worked pilots who were professionally interested in making high scores on the target meter showed more improvement on pregnenolone (average 25 per cent.) than did our paid civilian subjects (average 10 per cent.) who were not under as much working stress and whose interest in the experiments was less. In a third industrial group that we investigated in which there was no incentive pay pregnenolone did not increase production over placebo levels.

We believe that these findings to date are of a preliminary nature. The increase of human working efficiency without evidence of artificial overstimulation and untoward side effects is a desirable objective. We hope that others will be able to confirm us and advance this aspect of the field of biological engineering.

OBITUARY

HARRY FIELDING REID

DR. HARRY FIELDING REID, emeritus professor of dynamical geology and geography of the Johns Hopkins University, died on Sunday, June 18, just one month after his 85th birthday. Born in Baltimore, he took his A.B. and Ph.D. degrees at the Hopkins. After graduation he served successively as professor of mathematics and then of physics at Case School, returning to his alma mater in 1894 as lecturer, then as professor of geological physics, retiring in 1929.

His was a long and distinguished career as a scientist as evinced by his early election to the National Academy of Sciences. He was devoted to the precise thinking and rigid demands of proof which doubtless

[5] G. Pincus and H. Hoagland, *Jour. Aviation Med.*, 15: 98, 1944.