Table 1. Bioassay by the tibia test of growth hormone isolated from human, monkey, and beef pituitary glands.

Total dose (μg)	Beef		Monkey		Human	
	Rats (No.)	Tibia width (μ)	Rats (No.)	Tibia width (μ)	Rats (No.)	Tibia width (μ)
20	9	217 ± 5*	12	225 ± 5	8	213 ± 2
60	8	246 ± 2	13	248 ± 3	8	235 ± 2
120	8	268 ± 8	14	276 ± 6	6	256 ± 2

* Mean ± standard error.

Table 2. Physicochemical characteristics of growth hormone isolated from human, monkey, and beef pituitary glands.

Physicochemical characteristics	Beef*	Monkey	Human
Sedimentation constant, $S_{20,w}$	3.19 *S*	1.88† *S*	2.47† *S*
Diffusion constant, $D_{20} \times 10^7$	7.23	7.20†	8.88†
Molecular weight	46,000	25,400	27,100
Electrophoretic mobility‡ (cm^2/sec/volt)	6.8×10^{-5}	5.1×10^{-5}	
Isoelectric point, *p*H	6.85	5.5	(5.5)‖

* Taken from C. H. Li (*13*).
† Carried out in *p*H 2.3 phosphate buffer of 0.2 ionic strength.
‡ Acetate buffer of *p*H 4.0 and ionic strength of 0.03 at 0.5°C.
‖ *p*H of minimal solubility in salt-free solution.

Table 3. Amino acid composition of growth hormone isolated from human, monkey, and beef pituitary glands (No. of residues per mole).

Amino acid	Beef*	Monkey	Human
Glutamic acid	50	33	36
Aspartic acid	35	26	31
Cystine	4	4	2
Serine	22	20	20
Threonine	26	13	14
Glycine	20	15	14
Alanine	31	11	14
Proline	14	10	12
Valine	14	9	10
Methionine	7	6	4
Leucines	76	41	38
Phenylalanine	27	16	14
Tyrosine	11	7	5
Lysine	23	12	12
Histidine	7	5	5
Arginine	26	13	14
Tryptophan	3	1	1
Total	396	241	245

* Taken from Li and Chung (*14*).

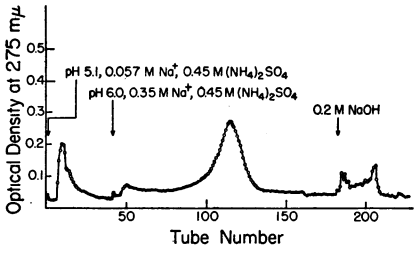

Fig. 1. Chromatography on the Na form of Amberlite XE-97 resin (dimension of column, 3 by 30 cm) of a growth hormone concentrate (100 mg) obtained from human pituitaries; 10 ml per tube. The hormonal activity is located in tubes 99 to 127.

then to *p*H 5.5; any precipitates formed at these two *p*H's were removed by centrifugation. The clear supernatant fluid was then diluted to a 0.2 percent solution; 40 percent ethanol (volume per volume) was added slowly at 0°C with vigorous stirring until the concentration of ethanol reached 5 percent by volume. The precipitate formed was removed by centrifugation and discarded; the supernatant was brought to 20 percent ethanol. The 5- to 20-percent ethanol precipitation was dissolved in a solution of *p*H 7 and lyophilized. The final product (*8, 9*) weighed 29 mg. By the same procedure, 20 mg of the somatotropin protein could be obtained from 1 g of lyophilized monkey pituitaries. These products, when assayed in hypophysectomized rats by the tibia test (*9*), were found to have growth-promoting activities comparable to that of the beef hormone, as shown in Table 1.

Both human and monkey somatotropin preparations have been submitted for purity studies employing electrophoresis and ultracentrifugation, as well as N-terminal amino acid analysis. These investigations indicate that both preparations possess a high degree of homogeneity. Certain physicochemical data may be seen in Table 2. Dinitrophenylation (*10*) of both human and monkey hormone protein yielded phenylalanine as the sole N-terminal residue. Amino acid analyses of human and monkey hormone preparations reveal that they are similar but that their compositions differ significantly from that of the beef hormone (see Table 3); tyrosine and tryptophan were estimated spectrophotometrically (*11*), and the other amino acids were estimated by quantitative paper chromatography of their dinitrophenyl derivatives (*12*).

It may be recalled that structural investigations (*13*) of growth hormone from beef pituitaries have shown that the hormone protein with a molecular weight of 46,000 appears to consist of a branched polypeptide chain having two N-terminal residues (phenylalanine and alanine) and only one C-terminal residue (phenylalanine). The findings reported here indicate that the human and monkey hormones are proteins with a molecular weight of approximately 26,000, with only one N-terminal residue (phenylalanine), and with isoelectric points more acidic than that of the beef hormone. Whether or not the human and monkey hormones, prepared by the procedure herein described, are effective in man is being investigated; the results of such studies will be reported at a later date.

CHOH HAO LI
HAROLD PAPKOFF
Hormone Research Laboratory and
Department of Biochemistry,
University of California, Berkeley

References and Notes

1. C. H. Li and H. M. Evans, *Science* 99, 183 (1944); C. H. Li, H. M. Evans, M. E. Simpson, *J. Biol. Chem.* 159, 353 (1945); *Science* 108, 624 (1948); A. E. Wilhelmi, J. B. Fishman, J. A. Russell, *J. Biol. Chem.* 176, 735 (1948).
2. L. L. Bennett *et al.*, *J. Clin. Endocrinol.* 10, 492 (1950); E. Shorr *et al.*, in *The Hypophyseal Growth Hormone, Nature and Actions,* R. W. Smith, Jr., O. H. Gaebler, C. N. H. Long, Eds. (Blakiston, New York, 1955), p. 522.
3. G. E. Pickford, *Endocrinology* 55, 274 (1954); A. F. Wilhelm, cited by G. E. Pickford.
4. E. Knobil and R. O. Greep, *Federation Proc.* 15, 111 (1956).
5. The data in this report were presented before the annual Scientific Session of the American Cancer Society in New York on 29 Oct. 1956. This work was supported in part by grants from the Albert and Mary Lasker Foundation and from the American Cancer Society.
6. We wish to thank Rolf Luft and Herbert Olivecrona of Stockholm, Sweden, who kindly put at our disposal the human pituitaries, and Otto K. Behrens of the Eli Lilly Laboratories for his generosity in supplying monkey pituitary glands.
7. C. H. Li, *J. Biol. Chem.* 211, 555 (1954).
8. C. A. Gemzell and F. Heijkenskjöld, (private communication; *Endocrinology* 59, 631 (1956)) have prepared a growth-hormone fraction from human pituitaries (fraction A of the published procedure, 7) and have found that this fraction has a biological activity, when tested in the rat (*9*), comparable to that of the beef hormone; these investigators concluded that each human pituitary contained growth-hormone activity equivalent to 3.6 to 6.0 mg of a reference beef hormone.
9. I. I. Geschwind and C. H. Li, in *The Hypophyseal Growth Hormone, Nature and Actions,* R. W. Smith, Jr., O. H. Gabler, C. N. H. Long, Eds. (Blakiston, New York, 1955), p. 28.
10. F. Sanger, *Biochem. J.* (*London*) 39, 507 (1945).
11. T. W. Goodwin and R. A. Morton, *ibid.* 40, 628 (1946).
12. A. L. Levy, *Nature* 174, 126 (1954).
13. For a summary, see C. H. Li, *Advances in Protein Chem.* 11, 101 (1956).
14. C. H. Li and D. Chung, *J. Biol. Chem.* 218, 33 (1956).

16 November 1956

Control of Drinking Behavior by Means of an Operant-Conditioning Technique

This paper (*1*) describes an operant-conditioning technique for forcing rats to ingest fluid in amounts far in excess of their normal requirements. Operant-conditioning techniques (*2*) have been used to train animals to obtain food or water or to avoid electric shock by performing certain arbitrary responses, such as pressing a lever. Once the animal is responding regularly, the frequency and the distribution in time of these responses can be manipulated by the use of various schedules of reinforcement (*3*). In the present experiment, the act of drinking was treated as operant behavior to be conditioned by means of procedures developed for responses such as bar-pressing. By controlling the frequency of the animal's drinking, the experimenter manipulates the amount ingested.

The rat is placed in a cage where intermittent shocks are delivered through

a grid floor. By licking the fluid at the end of a tube, the rat can postpone the next shock. The licks are detected by means of an electronic apparatus called a "drinkometer" (*4*): each time the rat's tongue touches the fluid, a thyratron tube operates the recording and programing apparatus.

We have found that drinking detected in this fashion can be reinforced successfully as operant behavior. Under a schedule of reinforcement, modified from one described by Sidman (*5*), we have succeeded in forcing ingestion of liquids that are normally refused, in producing ingestion of abnormally large quantities of water, and, using a liquid nutrient, in producing marked obesity in normal rats.

Careful selection and placement of the drinking tube are necessary to obtain these results. The tube is made of glass tubing 9 mm in diameter, flame-polished to an aperture of 3 mm at the lower end. The tube is mounted to form a 30° angle with the front of the cage, and an inverted 100-ml graduated cylinder, which serves as the fluid reservoir, is connected to the upper end. The lower tip of the tube is set 4 mm from a rectangular opening 5 mm wide by 9 mm high through which the rat licks. Restricting accessibility to the tube in this way insures that only the licking response is reinforced. Otherwise, alternative responses that operate the drinkometer (for example, pawing the tube or pressing it with the nose) may postpone the shock and tend to replace the drinking response.

Shock is provided by the output of a variable transformer connected to the grid floor. After each shock, the connections between the grid bars are automatically changed. This "grid-scrambling" was found necessary to prevent the rat from escaping shock by standing on bars continuously connected together. The duration of the shock pulse is 92 msec, and shocks are applied at 0.9 sec intervals if the rat does not respond.

Initial conditioning is best accomplished without strict limitation of the response. For this procedure, the drinking aperture resembles an inverted keyhole rather than a rectangle. The circular portion, 10 mm in diameter, allows the rat's jaw to protrude and brings the tongue nearer to the tube. During this phase of the training, the drinking tube is placed nearer to the aperture and filled with a 10-percent sucrose solution. Before being placed in the cage, the rats are deprived of water for 24 to 48 hours. Each lick postpones a 65-v shock for 5 sec. After a 12-hour period, the shock level is raised from 65 to 90 v.

A typical pattern of licking is generally observed when the shock level is raised: the rat licks once immediately after each shock, thereby postponing the next shock for 5 sec. Normally, in a situation where no shock avoidance is involved, rats drink in bursts of several hundred licks, and single licks rarely occur.

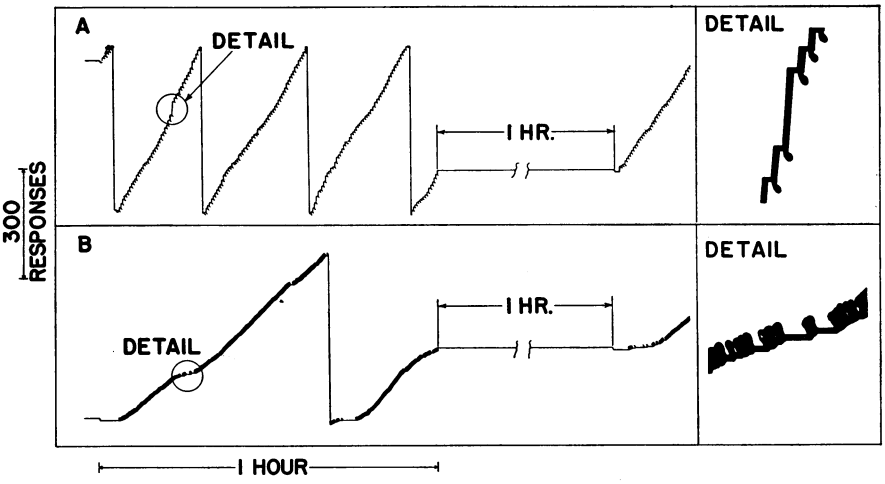

Fig. 1. Cumulative response records showing forced drinking during a typical 2-hr session. The pen moves upward with each lick and toward the right as time elapses. Each shock period is indicated by a small (⅛") downward displacement of the pen. (*A*) Drinking of a liquid nutrient showing several licks after each shock. (*B*) Drinking of a quinine solution showing single licks after each shock.

When experimental control of the licking response has been demonstrated in this manner, a schedule of reinforcement is selected that maintains drinking of appropriate quantities. Because single licks do not produce a large-volume intake, we developed a schedule that differentially reinforces several licks. The values of shock and the shock-postponement periods we chose are convenient for forcing the ingestion of a large quantity of liquid nutrient; other values may be appropriate for different liquids and when different durations of drinking are sought. Under our schedule of reinforcement, the rat can postpone shock for either a short period or a long one. The first lick following a shock postpones the next shock for 1 sec. Each successive lick results in the same postponement, unless three more licks are made within 7 sec of the first. In this case, the shock is postponed for 15 sec, and any lick within this 15-sec period, called the response-shock period, postpones the next shock for 15 additional sec.

Using these values, we were able to produce drinking of 10 ml per hour of a nutrient liquid (*6*) when drinking was reinforced on alternate hours for 24 hours each day. This procedure, continued for 20 days, resulted in the body weight's increasing from 240 to 406 g in a normal female rat. The average daily consumption of fluid in this period was 89 ml. When no shock is present, a normal female rat ingests about 48 ml daily and shows little or no gain in weight. Figure 1*A* shows a cumulative record of drinking during a 2-hour session. The short downward displacements indicate the onset of shock, and the recording pen remains in this displaced position until the next lick. It is readily seen that the occurrence of a shock is followed immediately by drinking sufficient to produce the longer postponement of shock. During the second hour, no shocks were presented. Likewise, no licking or drinking was observed. This typical result demonstrates effective control of drinking behavior.

If the shock sessions are carried out continuously for several hours, a change appears in the pattern of licking. The relative frequency of single licks increases until bursts of licking appear only rarely. We feel that this effect is due to the increasing aversion to drinking as the capacity of the stomach is approached.

Figure 1*B* was obtained with the same schedule of reinforcement, but with an aversive quinine solution substituted for the liquid nutrient. During a 24-hour period, the rats normally refused to drink the quinine solution ($3.4 \times 10^{-4} M$). During the period of forced drinking, the rat licked immediately each time the shock came on but never enough to produce the longer postponement period. The record for the next hour shows that no drinking occurred when shocks were not presented.

When water, rather than quinine or liquid nutrient, was placed in the drinking tube, six normal satiated animals drank approximately 30 ml in 5 consecutive hours under the same schedule of reinforcement.

The technique here described has been applied successfully to the problem of producing obesity in normal animals

by manipulating both the daily pattern of ingestion and the volume ingested. The technique appears useful when it is desirable to control fluid ingestion in normal satiated rats.

DAVID R. WILLIAMS*
PHILIP TEITELBAUM
Harvard University,
Cambridge, Massachusetts

References and Notes

1. This paper reports research supported in part by a grant from the National Science Foundation. We wish to thank William H. Morse of the Harvard University department of psychology for his advice and generosity in allowing the use of several pieces of control equipment.
2. B. F. Skinner, *The Behavior of Organisms* (Appleton-Century, New York, 1938).
3. C. B. Ferster and B. F. Skinner, *Schedules of Reinforcement* (Appleton-Century-Crofts, New York, in press).
4. E. Stellar and J. H. Hill, *J. Comp. Physiol. Psychol.* 45, 96 (1952).
5. M. Sidman, *ibid.* 46, 253 (1953).
6. The liquid diet is composed of 250 ml of evaporated milk, 125 ml of 50-percent sucrose solution, 150 ml of whole egg, 30 ml of Kaopectate, and 0.3 ml of Multi-Vi Drops (White Laboratories, Inc., Kenilworth, N.J.).

* Present address: Department of Psychology, Yale University, New Haven, Conn.

24 September 1956

Book Reviews

Statistical Mechanics, Principles and Selected Applications. Terrell L. Hill. McGraw-Hill, New York, 1956. 432 pp. Illus. $9.

It is always a very pleasant occasion when somebody who has made significant contributions to a field of physics can be persuaded to write a monograph on his field of interest, and the present monograph is no exception to the rule that usually such a monograph provides a welcome addition to the literature on the subject in question. The author discusses relatively briefly in the first three chapters the principles of statistical mechanics and the relation between statistical mechanics and thermodynamics. The fourth chapter deals with fluctuations. The fifth chapter treats the theory of imperfect gases and condensation, following largely Mayer's theory but giving also some new, alternative, derivations and discussing in the final section Yang and Lee's theory. The sixth chapter is devoted to a discussion of distribution functions and the liquid state. Chapter 7 deals with nearest neighbor lattice statistics, while the last chapter discusses lattice theories of the liquid and solid states. In a number of appendixes, the author gives some mathematical details. There is an adequate index at the back.

Although the author states that he hopes this monograph may be useful as a textbook for either an advanced course in statistical mechanics or as a supplement to a textbook such as Rushbrooke's in a more elementary course, I feel that the discussion is too technical for use as a textbook and that the main users of this monograph will be people working in the field who want to check up on the various methods that have been used in solving the problems discussed. For this purpose the monograph is an excellent one, and the discussion is very thorough.

I have a few minor criticisms, as one is always bound to have with any book. The reference to μ-space on page 92 is not really general in that it only refers to particles without internal degrees of freedom. The discussion of the third law of thermodynamics in section 14 is, to my mind, inadequate and does not pay sufficient attention to the clarification of the role of the third law which was given by Simon. Finally, I cannot feel that any useful purpose has been served by giving in 15 pages a bird's-eye view of quantum mechanics. Those readers who are familiar with quantum mechanics will know all that is contained in this section, but those who do not know sufficient quantum mechanics to use this monograph with any profit would certainly not be able to learn sufficient quantum mechanics from such a brief exposé. However, I would like to emphasize once again that these criticisms are only minor ones and that the over-all picture given by this book is a very pleasant one.

D. TER HAAR
Clarendon Laboratory, Oxford

Fundamental Concepts of Higher Algebra. A. Adrian Albert. University of Chicago Press, Chicago, Ill., 1956. 165 pp. $6.50.

For the greater part of his new book, A. A. Albert reworks the same ground treated in the first nine chapters of his *Modern Higher Algebra,* written nearly 20 years earlier. However, in the present volume his presentation leads up to a discussion of finite fields, whereas the former volume closed with an account of p-adic number fields.

The first chapter presents the elementary theory of finite groups; the second discusses rings, fields, and some basic concepts of ideals; and the third covers vector spaces and matrices. Chapter IV is devoted to finite algebraic extensions of a field and to Galois theory in the modern treatment. The fifth and final chapter applies the methods of the previous chapter in a systematic study of the irreducible polynomials over a finite field. A concluding section of Chapter V lists about 20 theorems of L. E. Dickson on finite fields, without giving proofs.

Although most of the book reviews the fundamentals of modern algebra, there is more here than a simple repetition of old material. The selection and arrangement are expertly done, and new proofs are produced for a number of theorems to improve the unity and logical structure of the presentation.

Unfortunately, the virtues of the book are likely to be appreciated only by the specialist. Albert's style at its softest makes few concessions to the reader, and in this case, as the author notes in his preface, "the presentation is extremely compact, and requires slow and careful classroom discussion," if it is to be used as a textbook for a first course in modern alegbra. A rather liberal sprinkling of typographic errors will add to the student's troubles.

A single example will show how compact the discussion is. The proof of Fermat's theorem (that when p is prime and does not divide a, $a^{p-1}-1$ is divisible by p) consists of the observation that the nonzero residue classes modulo p form a group of order p-1 under multiplicacation, together with a reference to the theorem that the order of a group is divisible by the order of any subgroup. Few authors would consider it superfluous to suggest at least that the subgroup to use is the one consisting of the powers of the residue class containing a.

It is too bad that the difficulties of style will limit the number of readers who might otherwise appreciate the brilliant qualities of this book. Mathematics could use more writers like G. H. Hardy.

WALTER JACOBS
Computation Division, U.S. Air Force