generally accompanied by sol-gel transformations, the possibility that the measured changes in protein —SH are due to this purely cytoplasmic event is eliminated in analyses of the anther.

The cycles of thiol concentrations associated with microspore mitosis are illustrated in Fig. 1. The solid line represents the total acid-soluble —SH and —SS— as determined by amperometric titration after electrolytic reduction of the extracts. It can be seen from the values for —SH alone (determined on the same extracts before reduction) that there is no conversion of —SS— to —SH preceding mitosis such as is postulated by Rapkine. Parallel determination of glutathione content of the extracts by the glyoxalase technique revealed the same cycle of variation as that found by the amperometric method. The soluble —SH was largely, though not entirely, glutathione.

The results are thus in agreement with the generalization of Rapkine that soluble thiol compounds increase prior to cell division. The source of such thiol is not, however, in a reservoir of soluble disulfide. On the contrary, there is an absolute increase in the concentration of glutathione preceding mitosis, and this high concentration persists until well after mitosis is completed. Variations of a smaller order of magnitude have been noted during the mitotic period, but because they have not yet been satisfactorily established they are omitted from the figure. Since formation and dissolution of the spindle occurs during mitosis and, since the high concentration of —SH extends well on both sides of the mitotic cycle, the idea that the concentration of glutathione varies directly as the degree of gelation of the spindle body cannot have general application. Indeed, by comparing the curves for protein and soluble —SH, respectively, it can be seen that in lily microspores the two components do not bear a reciprocal relationship to one another.

In one respect, the results obtained are consistent with earlier studies on marine eggs and protozoans: the association of an intense nitroprusside reactivity with the process of cell division. To this we can only add that the reactivity is, in fact, largely due to glutathione. There is no indication from the data concerning how the increase in soluble sulfhydryl occurs. The most probable explanation is that there is a synthesis of the compounds in question. Neither Rapkine's idea of protein as an —SS— reducing agent, nor Mazia's idea of the spindle as a glutathione-releasing agent can account for the behavior of the microscopes.

With respect to *function,* there are at least a few facts that point in the direction of a metabolic role for the glutathione. It has already been found in lily anthers that ascorbic acid concentration increases during microspore mitosis; by contrast, oxygen consumption falls (*7*, *8*). The twin occurrence of ascorbic acid and glutathione where the normal channels of terminal oxidation are interrupted may have some significance. Glutathione and ascorbic acid appear to play an important role in the respiration of embryonic plant tissues in which cell divisions are presumably frequent (*9*). In a number of animal tissues, ascorbic acid and glutathione have been found in isolated nuclei; possibly they are associated in some ways with nuclear metabolism (*8*). Thus, whether or not Rapkine was correct in picturing glutathione as effecting a fermentation in the cell to stimulate division, his idea that glutathione plays a metabolic role in the process of cell division certainly finds support in the behavior of lily microspores.

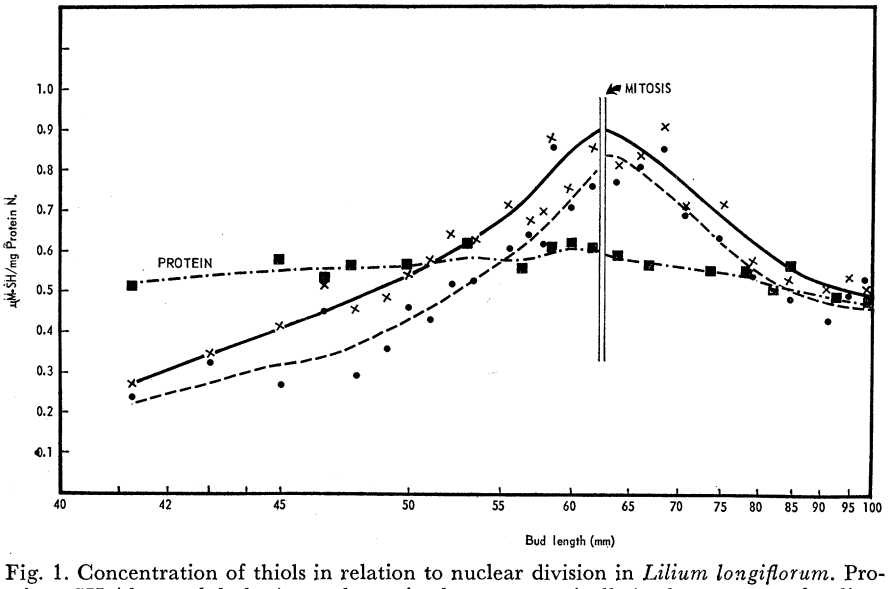

Fig. 1. Concentration of thiols in relation to nuclear division in *Lilium longiflorum.* Protein —SH (dots and dashes) was determined amperometrically in the presence of sodium lauryl sulfate after the protein had been washed twice with sulfosalicylic acid. The solid line represents total soluble thiols; the broken line, reduced soluble thiols. The use of bud length as an index of cell development has already been established (*7*).

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16 November 1956

Preparation and Properties of Growth Hormone from Human and Monkey Pituitary Glands

Following the isolation of growth hormone (somatotropin) in pure form from beef pituitaries (*1*), many attempts have been made to determine its effectiveness in man, but without success (*2*). Similarly, it has been shown that while growth hormone prepared from fish pituitaries is active in fish, it is not active in rats (*3*). Likewise, somatotropin concentrate from monkey pituitaries is active in the monkey, whereas the beef hormone is not (*4*). One of the obvious explanations for the failure of the beef somatotropin to act in man is that the beef somatotropin is chemically different from the hormone derived from man. We wish to report (*5*) that the human and monkey growth hormones are indeed different from the beef hormone.

From 0.8 g of lyophilized human pituitaries (*6*) extracted with CaO solution, an active concentrate was obtained by precipitation with 1.9*M* $(NH_4)_2SO_4$ as previously described (*7*). The $(NH_4)_2SO_4$ precipitate was extracted with phosphate buffer of *p*H 5.1, containing 0.057*M* Na^+ and 0.45 *M* $(NH_4)_2SO_4$. The clear extract was chromatographed on the polycarboxylic acid resin Amberlite IRC-50 (XE-97) under the conditions shown in Fig. 1. The contents of tubes 99 to 127 were combined, and the active component was precipitated by adding an equal volume of 5.0*M* $(NH_4)_2SO_4$. The precipitate was dissolved and dialyzed. The dialyzed solution was brought first to *p*H 4.5 and

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*).

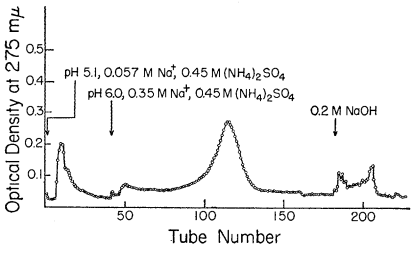

Fig. 1. Chromatography on the Na form of Amberlite XE-97 resin (dimension of column, 3 by 30 cm) of a growth hormone concentrate (100 mg) obtained from human pituitaries; 10 ml per tube. The hormonal activity is located in tubes 99 to 127.

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.

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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