warmed until the rectal temperature reached 36°C. This procedure required 30 minutes. During the warming, the second animal developed a spontaneous electrographic seizure localized to the right temporal cortex.

Both animals recovered without incident, and 24 hours later the repetitive stimulation was repeated. This was accomplished without anesthesia, while the animals were conscious and alert. In this condition, epileptiform activity was evoked with ease by stimulation with 20-v 60-cy/sec, 2.5-msec pulses. The clinical and electrographic effects of this stimulation were similar to those recorded before the application of hypothermia.

Finally, each animal was anesthetized, intubated, and immobilized by intravenous administration of succinyl chloride. Respiration was maintained by manual compression of the anesthesia bag. The Pentothal anesthetic was discontinued so that the animal was receiving oxygen by endotracheal tube and succinyl chloride by vein during artificial respiration.

Thirty minutes after the Pentothal had been discontinued, we began repetitive stimulation of the depth electrodes. Electrographic and clinical seizures occurred after stimulation with 12-v, 60-cy/sec, 2.5-msec pulses. The clinical seizures were confined to the face, and the electrographic tracings were quite similar to those obtained when the animal was in the unanesthetized state.

Figure 1 summarizes the electrographic recordings made at normal temperatures and under hypothermic conditions.

Actually, it was easy to establish afterdischarge by repetitive stimulation of the mesial temporal region at normal temperatures. Under the conditions of hypothermia, it was very difficult to do this.

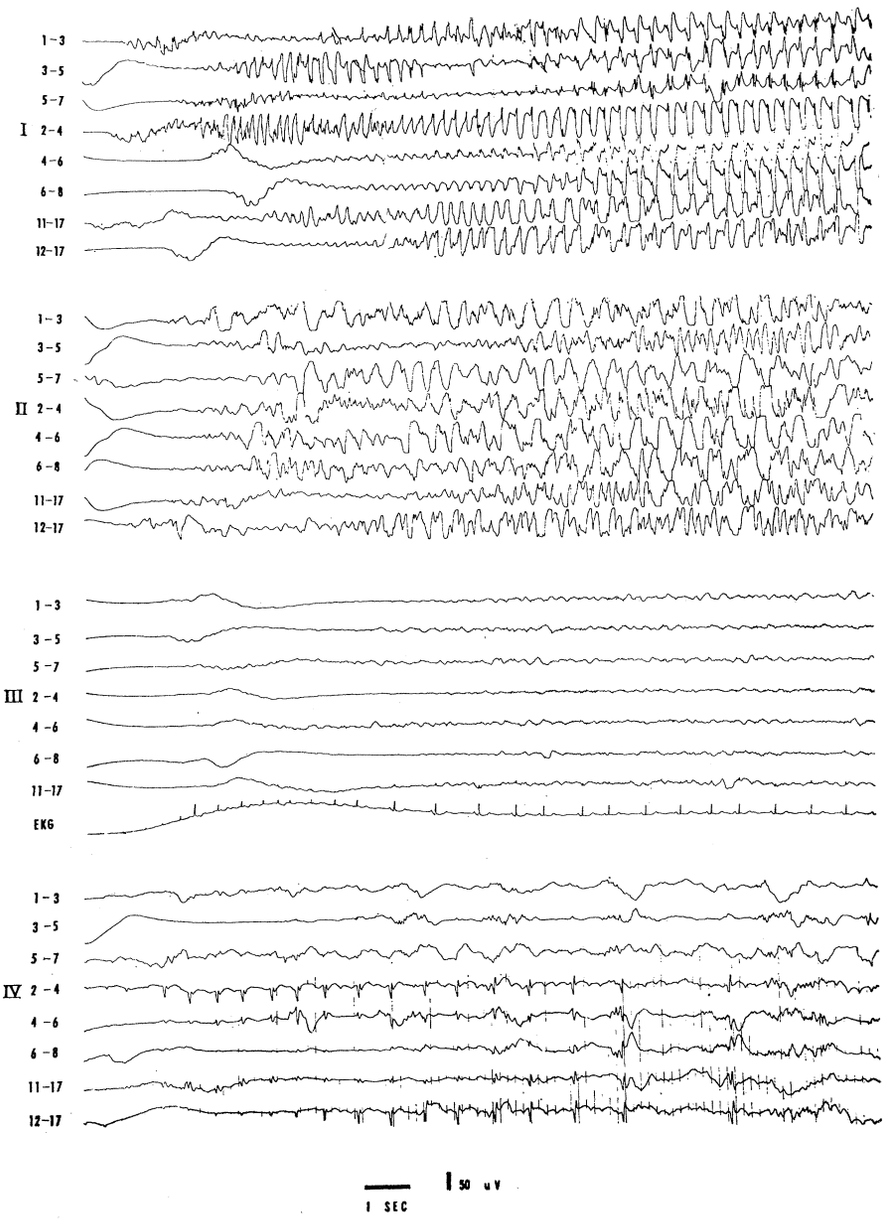

Fig. 1. Effect of hypothermia on epileptiform activity. Nos. 1 to 6, subdural electrodes on temporal cortex; Nos. 7 and 8, subdural electrodes on frontal cortex; Nos. 9 and 10, subdural electrodes over parietal cortex; Nos. 11, 12, and 17, needle electrodes inserted subcutaneously (i) over the midtemporal area (Nos. 11 and 12) and (ii) at the vertex at the midline (No. 17). Odd numbers indicate left side; even numbers, right side. Tracing I, generalized electrographic seizure in the freely moving animal following a stimulation at 10 v. Tracing II, generalized electrographic seizure in the anesthetized animal following a stimulation at 15 v. Normal temperature. Tracing III, no electrographic seizure in the anesthetized animal following stimulation at 70 v; temperature 30°C. Channel 8 is EKG. Tracing IV, localized seizure in the anesthetized animal following stimulation at 100 v; temperature 29.5°C.

Maitland Baldwin,
Laurence L. Frost,
Charles D. Wood, Shirley A. Lewis
National Institute of Neurological Diseases and Blindness, National Institutes of Health, U.S. Public Health Service, Bethesda, Maryland

References and Notes

1. W. K. Noell and S. A. Briller, Project No. 21-1202-0003, Rept. No. 1, Air University, U.S. Air Force School of Aviation Medicine, Randolph Field, Tex. (1953).
2. T. S. Githens, *J. Exptl. Med.* 18, 300 (1913); F. Grosse-Brockhoff and W. Schoedel, *Arch. exptl. Pathol. Pharmakol.* 201, 443 (1943).
3. We are indebted to Clarence Hebert and Delbert Dick for skillful application of anesthesia and hypothermia.
4. M. Baldwin, L. L. Frost, C. D. Wood, *Neurology*, in press.

26 September 1956

Suspected Correlation between Blood-Group Frequency and Pituitary Adenomas

The number of cases proving that the blood group genes are not selectively neutral is increasing rapidly. Since various summaries have been published recently, only our own findings will be reported here (*1*). Blood groups of patients in Boston hospitals with duodenal ulcer and carcinoma of the stomach were analyzed in a preliminary study, in order to determine whether the racially more heterogeneous American population would give deviations comparable to those found by various groups of British workers, beginning with Aird's (*2*) pioneering study.

The frequency of blood group A was found to be as much as 42.08 percent in patients with carcinoma of the stomach ($N = 663$), as compared with a frequency in the Massachusetts population

($N = 120,281$) of 39.7 percent, the latter based on the work of MacCready and Manin (*3*). In the sample of patients with duodenal ulcer ($N = 144$) the frequency of group O was found to be 61.11 percent, as compared with 45.8 percent in the Massachusetts population. Similar findings for an Iowa population have been published by Buckwalter *et al.* (*4*). Although the magnitude of the deviation from the control is not identical in these various investigations, the direction is the same in all cases. This indicates that deviation from neutrality can be demonstrated even in the racially heterogeneous North American population.

An opportunity arose to extend this research to patients with brain tumors and pituitary adenomas, owing to the encouragement and exceptionally effective assistance of several neurosurgeons (*5*).

An analysis of 637 cases of brain tumors (Table 1) shows that the ABO gene frequencies do not deviate to any marked extent from those of the Boston population. There is an indication in glioblastoma multiforme and in meningeoma of a slight excess of group B, but the samples and deviations are too small to yield data of statistical significance. The diagnosis was confirmed by a tissue analysis in all cases, except some of those recorded as "unclassified."

The distribution of the ABO blood group in chromophobe adenoma of the pituitary is interesting. After a consistent striking deviation from population average was found in three Boston hospitals, two New York hospitals were included in the survey. A considerable excess of group O was found in each of the five hospitals (Table 2). Likewise, there was a striking deficiency of group A in each of these samples, while levels of group B were normal in some and perhaps slightly excessive in others.

There are relatively few hospitals where brain tumors and pituitary adenomas are operated, and these few may draw their patients from considerable distances. Although the great majority of the tabulated cases were from the Boston, or New York areas, several patients were from as far away as South America. The sample of the Massachusetts population has, therefore, only limited usefulness as control. The sample of brain tumors (Table 1) was therefore chosen as control sample for the statistical analysis of the blood group frequencies in pituitary adenoma (Table 2). There is no indication that the population from which the pituitary-adenoma sample was drawn is different in any way from the population from which the brain-tumor sample was drawn. Chi square comparisons of pituitary adenoma (Table 2) with brain tumors (Table 1) yielded the following

Table 1. Distribution and frequency of ABO blood group in brain tumors in Boston hospitals.

Item	Group								Total N
	O		A		B		AB		
	N	%	N	%	N	%	N	%	
Glioblastoma multiforme	78	46.62	72	39.34	26	14.21	7	3.83	183
Astrocytoma	73	45.91	64	40.25	19	11.95	3	1.89	159
Ependymoma	9	50.00	8	44.49	1	5.56	0	0	18
Meningeoma	71	43.29	63	38.41	24	14.63	6	3.66	164
Acoustic Neuroma	26	44.07	24	40.68	7	11.86	2	3.39	59
Rare types*	12	48.00	10	40.00	2	8.00	1	4.00	25
Unclassified	13	44.83	11	37.93	2	6.90	3	10.34	29
Total brain tumors	282	44.27	252	39.56	81	12.72	22	3.45	637
Boston population	55,089	45.80	47,752	39.70	12,990	10.80	4450	3.70	120,281

* Medulloblastoma, Pinealoma, Oligodendroglioma.

Table 2. Distribution and frequency of ABO blood group in chromophobe adenoma of the pituitary and controls.

Item	Group								Total N	% increase in group O*
	O		A		B		AB			
	N	%	N	%	N	%	N	%		
Massachusetts General Hospital	11	61.11	3	16.67	4	22.22	0	0	18	33.43
N.E. Deaconess Hospital	22	62.86	8	22.86	4	11.43	1	2.86	35	37.23
Baptist Hospital	2	50.00	1	25.00	1	25.00	0	0	4	9.17
Total Boston hospitals	35	61.40	12	21.05	9	15.79	1	1.75	57	34.06
New York Hospital, N.Y.	12	52.17	3	13.04	7	30.43	1	4.35	23	13.91
Presbyterian Hospital, N.Y.	27	62.79	9	20.93	3	6.98	4	9.30	43	37.10
Total, pituitary adenoma	74	60.16	24	19.51	19	15.45	6	4.88	123	31.35
Total, brain tumors	282	44.27	252	39.56	81	12.72	22	3.45	637	
Boston population	55,089	45.80	47,752	39.70	12,990	10.80	4450	3.70	120,281	

* Over total, brain tumors.

results. Group O compared with the sum of the other blood groups (A + B + AB): $\chi^2 = 9.97$, $p = 0.0017$. Group A compared with the sum of the other blood groups (O + B + AB): $\chi^2 = 18.45$, $p = < 0.0001$. There is no significant deviation for B or AB. In view of the smallness of the total sample, it is advisable to consider these findings as tentative, in spite of the statistical significance of the deviation and the concordance of the data from four of the five hospitals (Table 2).

These findings raise some interesting questions. In most other previously established cases of a correlation between a pathological condition and blood groups, the intestinal tract (stomach, duodenum, pancreas) was involved directly or indirectly. With the pituitary, an endocrine gland is found to be concerned. What particular attributes of group O should make carriers of group O more and of group A less readily subject to abnormal growth of the chromophobe cells of the pituitary is a complete mystery. Yet the new findings are in line with the broad concepts of population genetics that genes have pleiotropic effects and may participate in a great many aspects of the phenotype. Since pituitary adenoma is a very rare condition, mortality due to this condition will not contribute much to depress the frequency of group O in the population. Particularly unbalanced blood group frequencies are perhaps more likely to occur in rare than in common diseases, although they are not absent in common ones (for example, duodenal ulcer and carcinoma of the stomach).

The present findings accentuate the problem of the rarity of group B in the European and North American population. It is the rarest gene of the ABO blood group, yet so far it has not been found to be discriminated against in a single pathological condition. What factor depresses group B to its low frequency is, as Aird (*6*) has pointed out to us, one of the great puzzles of the blood group field. Some childhood or infectious disease is most suspect.

ERNST MAYR
Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts

LOUIS K. DIAMOND
Children's Hospital, Boston, Massachusetts

R. PAUL LEVINE
Biological Laboratories, Harvard University

MARGARET MAYR
Museum of Comparative Zoology, Harvard University

References and Notes

1. This investigation was supported by research grant H2409 from the National Heart Institute, National Institutes of Health, U.S. Public Health Service. We deeply appreciate the many courtesies received during the course of this investigation, in particular the permission to study hospital and blood-bank records kindly given by many doctors and hospital officials, especially by J. C. White and M. G. Rasmussen of the Massachusetts General Hospital, G. Horrax of the Lahey Clinic, H. Kenton of the New England Deaconess Hospital, J. L. Pool of the Presbyterian Hospital, New York, and B. Ray of the New York Hospital, New York.
2. I. Aird, H. H. Bentall, J. A. Fraser Roberts, *Brit. Med. J. I* 1953, 799 (1953).
3. R. A. MacCready and M. C. Manin, *J. Lab. Clin. Med.* 37, 634 (1951).
4. J. A. Buckwalter *et al.*, *Science*, 123, 840 (1956).
5. We would especially like to thank Hannibal Hamlin and William Sweet.
6. I. Aird, personal communication.

16 August 1956

Isolation and Properties of Corticotropin from Bovine Pituitary Glands

The structural formulas that have been elucidated for ACTH preparations isolated from ovine (*1*) and porcine (*2, 3*) pituitary glands (α-corticotropin and corticotropin-A, respectively) have revealed that these preparations are not identical. A recent note by White and Peters (*4*) describes the results of preliminary physical and chemical studies on a bovine ACTH preparation; similarities in amino acid composition between the bovine preparation and porcine corticotropin-A, as well as an identity in the patterns of amino acids that were released when these two preparations were treated with enzymes, were noted. We would like to report (*5*) that in this laboratory an identical amino acid composition has been found for bovine ACTH as for the ovine (*6, 7*) product (α-corticotropin), and in addition, that the hormones of these latter two species manifested identical behavior in resin column chromatography and in countercurrent distribution. Hence, the properties of bovine ACTH would seem to be closer to those of the ovine than to those of the porcine hormone.

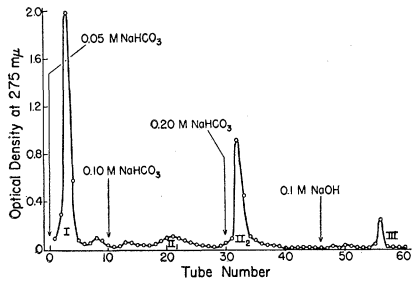

Fig. 1. Chromatography on the Na form of Amberlite XE-97 resin (dimensions of column, 0.9 by 24 cm) of an ACTH concentrate (20 mg) obtained from beef pituitary glands; 3 ml per tube. The hormonal activity is located in peaks II_1 and II_2.

The bovine corticotropin was isolated from whole beef pituitaries by the same procedure previously described for the hormone from sheep glands (*6, 7*), except for omission of the step involving zone electrophoresis on starch. The chromatographic pattern of the concentrate obtained at the dioxane step on the Amberlite IRC-50 (XE-97) column may be seen in Fig. 1; the activity was found in peaks II_2 and II_1. It may be noted that the positions of these two active peaks are identical with those obtained (*7*) with the ovine ACTH concentrate. The material in peak II_2 was desalted and submitted to 200 transfers in an all-glass countercurrent distribution apparatus (*8*) in a 2-butanol/0.2-percent trichloroacetic acid system (Fig. 2). The material in those tubes falling within the theoretical distribution curve for a partition coefficient (K) of 1.06 was found to be active (*9*), and it behaved as a single substance when it was submitted to terminal amino acid analyses. It may be recalled that corticotropin-A (of porcine origin) distributes in the 2-butanol/0.2-percent trichloroacetic acid system with a K value of 1.82 (*10*), whereas α-corticotropin in the same solvent system distributes with a K value of 1.0 (*11*).

The molar ratios of amino acids in the bovine hormone are as follows: alanine, 3; arginine, 3; aspartic acid, 2; glutamic acid, 5; glycine, 3; histidine, 1; leucine, 1; lysine, 4; methionine, 1; phenylalanine, 3; proline, 4; serine, 3; tryptophan, 1; tyrosine, 2; and valine, 3. Tyrosine and tryptophan were estimated spectrophotometrically (*12*), while the other amino acids, including tyrosine, were estimated by quantitative paper chromatography of their dinitrophenyl derivatives (*13*). It can be noted that these values are identical with those found for α-corticotropin (*14*). Earlier investigations (*2, 6, 14*) showed that there is a difference in amino acid composition between the peptide hormones isolated from sheep and from pig glands —namely, one more serine and one less leucine in the former.

N-terminal amino acid anaylsis of the bovine hormone by means of both the fluorodinitrobenzene and phenyl*iso*thiocyanate procedures (*15*) disclosed serine as the sole terminal residue. The paper-strip modification (*16*) of the phenyl*iso*-thiocyanate method yielded the following N-terminal sequence for bovine corticotropin: serine, tyrosine, serine, methionine. . . . The amino acid released from the carboxyl end of the peptide hormone obtained by the carboxypeptidase procedure (*17*) was phenylalanine. Thus, with respect to N-terminal amino acid sequence and the C-terminal residue, the hormones of all three species are identical (*1–3, 18*).

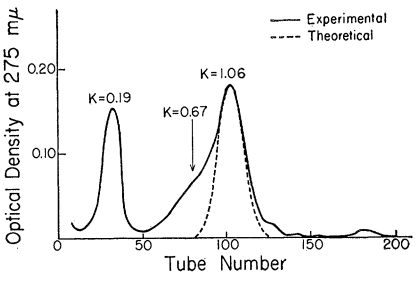

Fig. 2. Countercurrent distribution (200 transfers) of material (77 mg) obtained from chromatography on an XE-97 resin column (see Fig. 1); system, 2-butanol/0.2-percent aqueous trichloroacetic acid. The component with $K = 0.6$ is the bovine corticotropin.

The findings reported here indicate an identity between bovine ACTH and α-corticotropin, but the final proof for this conclusion must await the elucidation of the structural formula of the bovine hormone. Such structural studies are now being carried out and will be reported in a subsequent paper.

CHOH HAO LI
JONATHAN S. DIXON
Hormone Research Laboratory and
Department of Biochemistry,
University of California, Berkeley

References and Notes

1. C. H. Li *et al.*, *Nature* 176, 687 (1955).
2. P. H. Bell, *J. Am. Chem. Soc.* 76, 5565 (1954).
3. W. F. White and W. A. Landmann, *ibid.* 77, 1711 (1955).
4. W. F. White and R. L. Peters, *ibid.* 78, 4181 (1956).
5. This report is paper XI of the corticotropins (ACTH) series. This work was supported in part by grants from the Albert and Mary Lasker Foundation and from the National Institutes of Health of the U.S. Public Health Service (grant No. G 2907).
6. C. H. Li *et al.*, *Nature* 173, 251 (1954).
7. C. H. Li *et al.*, *J. Biol. Chem.* 213, 171 (1955).
8. L. C. Craig and H. O. Post, *Anal. Chem.* 21, 500 (1949).
9. From 1 kg of whole beef pituitaries, the yield of the hormone averaged about 8 mg. When the material was assayed in hypophysectomized rats, it possessed an ACTH potency similar to that of α-corticotropin. Like the sheep hormone, the corticotropin from beef pituitaries has a me'anocyte-stimulating activity.
10. W. F. White, *J. Am. Chem. Soc.* 75, 503 (1953).
11. The value reported in our early investigations for the partition coefficient in this solvent system was 0.72. For reasons we do not understand, the K value found recently has been consistently 1.0. Furthermore, in all recent experiments, bovine and ovine preparations have distributed with the same partition coefficient when they have been submitted to countercurrent distribution in the same solvent system.
12. T. W. Goodwin and R. A. Morton, *Biochem. J.* (*London*) 40, 628 (1946).
13. A. L. Levy, *Nature* 174, 126 (1954).
14. A. L. Levy, I. I. Geschwind, C. H. Li, *J. Biol. Chem.* 213, 187 (1955).
15. F. Sanger, *Biochem. J.* (*London*) 39, 507 (1945); P. Edman, *Acta Chem. Scand.* 4, 283 (1950).
16. H. Fraenkel-Conrat, *J. Am. Chem. Soc.* 76, 3606 (1954).
17. J. I. Harris *et al.*, *J. Biol. Chem.* 209, 133 (1954).
18. J. I. Harris and C. H. Li, *J. Am. Chem. Soc.* 76, 3607 (1954).

21 September 1956