

TABLE I.

No. of Compound	Barbituric Acid	M.H.D. mg. per kg.	M.A.D. mg. per kg.	M.L.D. mg. per kg.	Average Duration of Action min.	Therapeutic Index: M.L.D./M.A.D.
1	2,4-dimethyl-pentyl-ethyl-sodium salt	50	70	140	63	2.00
2	1,4-dimethyl-pentyl-ethyl-sodium salt	60	80	240	69	3.00
3	1-propyl butyl-ethyl-sodium salt	20	30	65	75	2.16
4	1,3-dimethyl-pentyl-ethyl-	50	70	200	102	2.85
5	4-methyl-heptyl-ethyl-	60	80	230	115	2.87
6	2,4-dimethyl-hexyl-ethyl-	60	80	230	132	2.87
7	2-ethyl-hexyl-ethyl-sodium salt	50	80	230	154	2.87
8	4-methyl-pentyl-ethyl-	50	70	180	155	2.57
9	5-methyl-2-ethyl-hexyl-ethyl-	100	140	340	158	2.42
10	2-methyl-pentyl-ethyl-	50	70	180	225	2.57
11	3-methyl-2-ethyl-hexyl-ethyl-	120	160	400	255	2.50
12	1,3-dimethyl-butyl-ethyl-	none*	none*	10*	—	—

\*Convulsions.

## 7394 C

### A Procedure for Determining Distribution of Blood Groups in Mummies.\*

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Some unexpected differences in the distribution of blood groups observed by us a year ago<sup>1, 2</sup> among the "Blackfeet" and "Blood" tribes of American Indians suggested that the different tribes may

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<sup>1</sup> Matson, G. Albin, *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1380.

<sup>2</sup> Matson, G. A., and Schroder, H. F., *J. Immunol.*, 1933, **25**, 155.

not have had a single origin and that at least some of the tribes may not have separated from the rest of the human family before the A agglutinin developed in the race. Since the possibility of a degree of racial admixture among putative full blooded Indians always remains, regardless of their pedigree as given in the Agency records, the desirability of detecting the A agglutinin in mummies of Indians who lived before the advent of the white man suggested itself.

Through the courtesy of Drs. G. D. Williams and H. A. McCordock, muscle tissue from 12 mummies from Peru, Arizona and Greenland, was placed at our disposal for such a study.

Because of the limited amount of material available in the preliminary experiments, dried cadaver tissues which had been previously washed and dried rapidly under a current of warm air were used in place of the mummy material.

The procedure followed at first was one in which O( $\alpha\beta$ ) serum was absorbed with cadaver tissues of groups O and A respectively by allowing mixtures of a small amount of the powdered tissue and varying dilutions of O( $\alpha\beta$ ) serum to remain over-night in the refrigerator. After centrifuging, the supernatant fluid was tested with O, A and B cells to determine whether the absorption of the agglutinins by the tissues had occurred. In such a test it is considered that if the group A cells are not agglutinated while B cells are, that the  $\alpha$  agglutinin in the O( $\alpha\beta$ ) serum has been absorbed by an A agglutinin in the tissue; conversely, if B cells are not agglutinated whereas A cells are, the tissue would be considered to have contained the B agglutinin which absorbed the  $\beta$  agglutinin from the O( $\alpha\beta$ ) serum. If, however, there is no inhibition of agglutination of either A or B cells, it may be assumed that the tissue contains neither A nor B agglutinin and may consequently be considered to belong to group O.

This procedure gave at times some confusing results, most likely due to the non-specific absorption by the particulate tissue material. To obviate this difficulty attempts were made to extract the isoagglutinin from the pulverized tissues and to use the extracts rather than the tissues themselves as a source of agglutinin.

These extracts of cadaver muscle tissue were made by mixing 1 gm. of powdered skeletal muscle tissue with 5-10 cc. of physiological saline solution and subjecting the mixture to repeated freezing and thawing. The tubes were then centrifuged and tests set up with the supernatant fluid. Varying amounts of the supernatant fluid (0.5 cc., 0.3 cc., 0.1 cc., and 0.05 cc.) were mixed with

TABLE I.  
Titration of residual agglutinins after absorption of O( $\alpha\beta$ ) serum with cadaver and mummy tissues.

Tissue Extract	Serum Dilutions														
	1:2			1:4			1:8			1:16			1:32		
	O	A	B	O	A	B	O	A	B	O	A	B	O	A	B
I M 1	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
I M 2	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
I M 3	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
I M 4	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
I M 5	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
I M 6	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
I M 7	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
I M 8	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
I M 9	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
I M 10	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
I M 11	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
I M 12	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
Cadaver Group O	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
Cadaver Group A	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—
NaCl Control	—	+++	+++	+++	+++	+++	—	+++	+++	+++	+++	—	+++	—	—

TABLE II.  
Titration of residual agglutinins after absorption of cow serum with cadaver and mummy tissues.

Tissue Extract	Serum Dilutions											
	1:2		1:4		1:8		1:16		1:32		1:64	
	O	Cells	O	Cells	O	Cells	O	Cells	O	Cells	O	Cells
I M 1	—	—	—	—	—	—	—	—	—	—	—	—
I M 2	—	—	—	—	—	—	—	—	—	—	—	—
I M 3	+	+	—	+	—	—	—	—	—	—	—	—
I M 4	+	+	—	—	—	—	—	—	—	—	—	—
I M 5	+	+	±	—	—	—	—	—	—	—	—	—
I M 6	+	+	—	—	—	—	—	—	—	—	—	—
I M 7	+	+	—	—	—	—	—	—	—	—	—	—
I M 8	+	+	±	—	—	—	—	—	—	—	—	—
I M 9	+	+	—	—	—	—	—	—	—	—	—	—
I M 10	+	+	—	—	—	—	—	—	—	—	—	—
I M 11	+	+	±	—	—	—	—	—	—	—	—	—
I M 12	+	+	—	—	—	—	—	—	—	—	—	—
Cadaver Group O	+	+	—	—	—	—	—	—	—	—	—	—
Cadaver Group A	+	+	+	+	+	+	+	+	+	+	+	+
NaCl Control	+	+	+	+	+	+	+	+	+	+	+	+

+, ++, +++, and ++++ = varying degrees of agglutination.  
— = no agglutination.

0.5 cc. of increasing dilutions of  $O(\alpha\beta)$  serum, the mixtures were allowed to remain over-night in the refrigerator and were tested the following day for the residual agglutinins with O, A, and B cells as above.

The results of this procedure were quite satisfactory. Group O cadaver tissue absorbed neither  $\alpha$  nor  $\beta$  from the  $O(\alpha\beta)$  serum, whereas group A tissues absorbed  $\alpha$  agglutinin. We were unable to obtain group B cadaver tissue and therefore this was not tried.

This procedure was then applied to the study of the mummy tissues. The results given in Table I indicate that neither  $\alpha$  nor  $\beta$  agglutinins were absorbed with mummy extracts nor by the group O cadaver tissue extract.

The question still remained, however, whether the mummy tissue left the  $\alpha$  and  $\beta$  agglutinins intact in  $O(\alpha\beta)$  serum by virtue of the absence of A and B agglutigen in the tissue (if tissues belonged to group O), or because the isoagglutinogens, if present in the tissue, had not been extracted.

To answer this question use was made of the known property<sup>3, 4, 5</sup> of cow serum, previously absorbed with  $A_1B$  human cells, to agglutinate specifically human blood cells of Group O. As the results in Table II show, the specific anti-O agglutinin of such serum was absorbed by the group O cadaver tissue extract and by the mummy tissue extracts examined, whereas there was no such absorption by the group A cadaver tissue extract.

While the findings here presented are insufficient to permit one to draw a conclusion as to the original distribution of the blood groups among the American Indians, this requiring a much larger series of specimens from different sources, the findings are recorded nevertheless because they offer a procedure which may be of value in further study of similar material from other localities.

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<sup>3</sup> Schiff, F., *Klin. Wochenschr.*, 1927, **6.1**, 303.

<sup>4</sup> Greenfield, G., *Z. f. Immunitätsf.*, 1928, **56**, 107.

<sup>5</sup> Sasaki, H., *Z. f. Immunitätsf.*, 1932, **77**, 101.