# Kinship of Smenkhkare and Tutankhamen affirmed by Serological Micromethod

A new serological micromethod has been used to show that the XVIIIth dynasty pharaohs Smenkhkare and Tutankhamen both belong to the same blood groups $A_2$ and MN.

## Microdetermination of Blood Group Substances in Ancient Human Tissue

THE extra-vascular occurrence of the ABH blood group substances and their resistance to degradation over very long periods of time has allowed us to determine the ABO blood groups of Egyptian and South American mummified remains. The inhibition of agglutination technique[1] normally used for palaeoserological studies requires relatively large amounts of tissue debris, usually of the order of 1 g[2], but studies in this department of the Tutankhamen remains, of which only very small amounts of tissue-dust were available, made necessary the development of a micromethod for detection of specific blood group substances.

About 10 mg tissue dust was pulverized in a ground-glass mill and shaken gently in 2 ml. Alsever's solution[3] for 24 h at 19° C, then centrifuged. To the supernatant fluid was added 0·1 ml. of a 0·1 per cent packed suspension of fresh human group O erythrocytes in Alsever's solution, the erythrocytes having been carefully washed four times in 10 ml. volumes of this solution. Before addition of the erythrocyte suspension, it was found advisable to test a sample of supernatant with a small volume of erythrocytes for any change in the osmotic pressure exerted due to elution of salts and other substances from the tissue and if necessary to adjust to physiological conditions with distilled water. The final suspension of erythrocytes was incubated at 37° C with intermittent gentle agitation for 1 h after which it was centrifuged and the erythrocytes again washed four times in 10 ml. volumes of Alsever's solution, and then re-suspended in 1 ml. of normal saline. This suspension was AB grouped in the usual manner using the tube technique with standard anti-A and anti-B sera and examined for agglutination microscopically.

Any ABH substances present in the tissue extract, being polysaccharides, are directly absorbed on to the red cell surface and can be readily detected by microscopic examination for agglutination after treatment with the appropriate antisera. The A groups, B and AB can be detected in this way, but, as with the inhibition technique, absence of both A and B substances cannot be assumed to indicate a group O origin for the tissue. Difficulties have been encountered with control material of known ABO groups using Ulex for detection of H substances by this technique, but it has been applied successfully to the detection of MN antigens. Attempts to detect Rh antigens in both fresh and ancient material with this technique have repeatedly produced only non-specific agglutination, unrelated to the Rh group in the case of the fresh tissue.

Detailed findings on the blood groups of the ancient material under examination are published below.

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[1] Landsteiner, K., and Richter, M., *Z. Medizinal. Beatme.*, **16**, 85 (1903).
[2] Matson, G. A., *J. Immunol.*, **30**, 459 (1936).
[3] Alsever, J. B., and Ainslie, R. B., *NY State J. Med.*, **41**, 126 (1941).

## Kinship of Smenkhkare and Tutankhamen demonstrated Serologically

RECENT researches[1] have demonstrated that the human remains in the Museum of Antiquities in Cairo, formerly thought to belong to Akhenaten (Amenophis IV), are more likely to be identified with Smenkhkare, co-regent and successor of Akhenaten as pharaoh of Egypt in the XVIIIth dynasty.

Because of the suggestions that Smenkhkare and Tutankhamen were brothers[2], and because the remains of Smenkhkare show several interesting anthropological features, it was important to re-appraise the anatomy of Tutankhamen, and this was done in December 1968. Detailed findings will be published elsewhere and it is sufficient here to note that there are very many points of similarity. Indeed, certain anthropometric measurements are identical. In order to investigate further the degree of kinship between these two pharaohs, it was clearly essential to estimate their blood groups.

It is well known that the ABH blood group substances occur not only on the red blood corpuscles but are also widely distributed throughout the tissues of the living body. The polysaccharide nature of these substances renders them highly resistant to climate and microbial degradation and they can be readily demonstrated in even very ancient human remains. It appears that the same can be said for the distribution and persistence of the MN substances but probably not for the antigens of the Rhesus system.

The sensitive technique developed in this department and described above has revealed blood group substances of the ABH and MN systems in tissue from Tutankhamen, Smenkhkare and other mummified remains. At present, it has not been possible to demonstrate with certainty any Rhesus antigens from mummified material.

The results of these investigations suggest that both Tutankhamen and Smenkhkare are blood group $A_2$ and that they are both MN. It is considered that these results are specific to the blood groups of the individuals concerned and not to microbial contamination for instance.