Table 1. Lymphoblast Transformation after 6 Days' Culture of Lymphocytes from a Smallpox-vaccinated (*B*) and a Non-vaccinated (*A*) Donor

Total cells counted on each slide were from 2 to 6×10^3

Lymphocytes from donor	Plasma from donor	Vaccinia virus	PHA*	Lymphoblasts (%)	Mitoses (%)	Tritiated cells (%)
B	*B*	+	−	3·67	0·29	1·34
B	*A*	+	−	3·68	0·42	1·40
B	*B*	−	−	0·03	0	0
B	*A*	−	−	0	0	0
A	*A*	+	−	0	0	0
A	*B*	+	−	0	0	0
A	*A*	−	−	0·03	0	0
A	*B*	−	−	0·05	0	0·02
A	*A*	−	+	70	+	Not done

Subject *B*, male, 32, vaccinated against smallpox.
Subject *A*, female, 24, never vaccinated against smallpox.
+, Large numbers of mitoses present.
* PHA cultures were harvested after three days.

Table 2. Lymphoblast Transformation after 6 Days' Culture of Lymphocytes from Mantoux Positive (*B*) and Mantoux Negative (*C*) Donors

Total cells counted on each slide were 2 to 6×10^3

Lymphocytes from donor	Plasma from donor	Old tuberculin	PHA*	Lymphoblasts (%)	Mitoses (%)	Tritiated labelled cells (%)
B	*B*	+	−	11·8	0·33	8·43
B	*C*	+	−	10·7	0·26	3·49
B	*B*	−	−	0·03	0	0·02
B	*C*	−	−	0·05	0	0·02
B	*B*	−	+	70	+	Not done
C	*B*	+	−	0	0	0
C	*C*	+	−	0·05	0	0·01
C	*B*	−	−	0·02	0	0·02
C	*C*	−	−	0	0	0·016
C	*C*	−	+	70	+	Not done

Subject *B*, male, 32, Mantoux positive with 1/1,000 old tuberculin.
Subject *C*, child aged 9, Mantoux negative with 1/100 old tuberculin.
+, Large numbers of mitoses present.
* PHA cultures were harvested after 3 days.

cellular deposit from the leucocyte rich plasma was washed four times with 0·15 M sodium chloride, pre-heated to 37° C, the cells being deposited after each washing by centrifuging at 250*g* for 5 min. A total white cell count was made after the final washing. The tissue culture medium was made by adding 0·4 ml. of the subject's plasma to 1·6 ml. of tissue culture 199 (ref. 4) containing 200 U penicillin per ml. and 100 μg streptomycin per ml. in 1 × 8 cm capped polystyrene tubes. Leucocytes from the appropriate subject were added to a final concentration of $1{\cdot}2 \times 10^6$ cells per ml. of culture medium. Old tuberculin to a final concentration of 1/4,000, the contents of one vial of heat-inactivated smallpox vaccine or 0·1 ml. of PHA were added to the cultures as indicated in Tables 1 and 2. The tubes were sealed and laid horizontally in an incubator at 37° C for three days. They were centrifuged, the supernatant discarded and replaced with fresh culture medium. The appropriate antigen or PHA was added in the same concentration as previously and the culture tubes sealed and laid horizontally at 37° C for a further 3 days. On the final day, 20 μg of desacetylmethycolchicine ('Colcemid', Ciba, Ltd.) in 0·1 ml. was added to each culture. After 3 h incubation, 1 μg of tritiated thymidine in 0·05 ml. (thymidine (methyl-*T*), specific activity 3 C. mole) was added to each tube and the cultures reincubated for 1 h. After centrifuging at 250*g* for 5 min, the supernatant was discarded and several smears of the cellular deposit were made on gelatin-coated slides. After drying, one slide from each culture was stained with May–Grünwald–Giemsa. Autoradiography was performed on another slide using Kodak '*AR* 10', exposed for six days and counterstained after development with periodic acid–Schiff stain. The cellular detail of the smears was studied at a magnification of 1,200 times. An attempt was made to count and identify all the cells on each smear. These were of different cellular densities and yielded total cell counts from 2 to 6×10^3 cells per slide.

After six days of culture most of the cells were lymphocytes. Occasional eosinophils were seen, but no neutrophils were recognized. Lymphoblasts were recognized as cells over 15μ diameter which when stained with May–Grünwald–Giemsa had large purple nuclei containing 2 or 3 nucleoli and with varying amounts of light blue cytoplasm of 'frosted-glass' appearance often containing several vacuoles. Mitotic figures arrested in metaphase were seen in cells of the same order of size as lymphoblasts and with similar cytoplasmic features. In the autoradiographs, cells were accepted as being labelled if they contained more than twenty silver grains over the nucleus.

The degree of lymphoblast transformation, mitotic activity and uptake of tritiated thymidine is shown in Tables 1 and 2.

These results show that old tuberculin and heat-killed vaccinia virus induce lymphoblast transformation of peripheral blood lymphocytes from an immune donor in the absence of autologous plasma. They also demonstrate that the plasma from an immune individual does not confer the potential for lymphoblastic transformation *in vitro* in the presence of antigen to lymphocytes from a non-immune individual. This finding is consistent with the ability to transfer delayed-type tuberculin sensitivity *in vivo* with lymphocyte suspensions and not with plasma[5].

In these experiments, PHA stimulated lymphoblast transformation in the absence of autologous plasma. The considerable quantitative differences between the dynamics of lymphoblast transformation caused by antigens and that caused by PHA suggest that PHA acts as a direct cellular stimulant and not as an antigen[6]. This is supported by our recent finding that PHA stimulates mitosis in epithelial cells of adult human skin grown in an organ culture system *in vitro*[7]. The fact that the plasma from sensitized individuals does not contain any component which can induce the transformation of lymphocytes from unsensitized individuals into lymphoblasts in the presence of specific antigen would indicate the probability that cytophilic antibodies[8] were not involved in this reaction.

We thank Mr. P. Gaylard for technical help and the Herbert E. Dunhill Trust for support.

G. A. Caron
I. Sarkany

Department of Dermatology,
Royal Free Hospital,
London.

[1] Nowell, P. C., *Cancer Res.*, **20**, 402 (1960).
[2] Pearmain, G., Lycette, R. R., and Fitzgerald, P. H., *Lancet*, i, 637 (1963).
[3] Cowling, D. C., Quaglino, D., and Davidson, E., *Lancet*, ii, 1091 (1963).
[4] Morgan, J. F., Morton, R. J., and Parker, R. C., *Proc. Soc. Exp. Biol.*, **73**, 1 (1950).
[5] Lawrence, H. S., *Proc. Soc. Exp. Biol. and Med.*, **71**, 516 (1949).
[6] Grasbeck, R., Nordman, C., and de la Chapelle, A., *Lancet*, ii, 385 (1963).
[7] Sarkany, I., and Caron, G. A., *Brit. J. Dermatol.*, **77**, 439 (1965).
[8] Boyden, S. V., in *Cell Bound Antibodies*, edit. by Amos, B., and Koprowski, H., 7 (The Wistar Institute Press, Philadelphia, 1963).

Distribution of Haptoglobin Types in Turkish People

Since the discovery by Smithies that haptoglobins show three distinct patterns in starch-gel electrophoresis, the interest in these α-2- globulins has become widespread[1]. This property has an immediate application in anthropological and genetic investigations, and many investigations were carried out to determine the distribution of haptoglobin types in various populations and ethnic groups. It is well known that blood-group frequencies were previously used for such studies. But, as will be discussed later, haptoglobin typing proved more accurate in this regard.

In the present investigation, the distribution of haptoglobin types has been investigated in sera obtained from 300 Turks. Some of these people were inhabitants of Istanbul while others came from different regions of Anatolia. The samples were obtained from the patients at the Çapa Internal Clinic of the Istanbul Medical School and from the patients referred to the biochemical laboratory of this clinic for biochemical determinations. The

©1966 Nature Publishing Group

Table 1. Distribution of Haptoglobin Types in Turkish People

No. tested	Distribution of haptoglobin types (per cent)					Gene frequency	
	Type 1–1	Type 2–1	Type 2–2	Type 2–1M	Type Johnson	Hp[1]	Hp[2]
300	8	46	45·1/3	1/3	1/3	0·31	0·68

haptoglobin types were determined by the vertical starch-gel electrophoretic method of Smithies[2]. The position of the haptoglobin bands was determined by staining with orthodianisidine[3]. The results of these investigations are presented in Table 1.

The distribution of the major haptoglobin types is quite uniform in populations living in different European countries : Hp 1-1, 14–17 per cent ; Hp 2-1, 47–49 per cent ; and Hp 2-2, 36–40 per cent[4–12]. Gene frequencies for Hp[1] and Hp[2] are also very similar : 0·36–0·40 and 0·60–0·66 respectively[4–12]. On the other hand, in Africa, Hp 1-1 is much more frequent (49–54 per cent), and the gene frequency of Hp[1] is much higher (0·60–0·72) (refs. 13–15). In Asia, on the contrary, the percentage of type 1-1 is lower (5–10 per cent) and so is the gene frequency of Hp[1] (0·21–0·31) (refs. 16–19).

As can be seen from Table 1, the distribution of major types in Turkish people is similar to that seen in Asiatic countries. This is not surprising, considering the historical fact that the Turks emigrated for several centuries from Central Asia to Asia Minor. On the other hand, a survey of different blood-group frequencies (ABO–MNS, Rh, Lutheran, Kell and Duffy) in Turkish people[20] showed that they did not differ greatly from the values commonly found in Europe. Such a discrepancy also occurred in the case of the Bushmen of Southern Africa. Although their physical appearance suggested a Mongoloid ancestry, blood-group studies performed by Weiner *et al.*[21] enabled them to conclude that the blood group frequencies of Bushmen showed a negroid pattern. But the distribution of haptoglobin types in Bushmen was different from other Africans and similar to the distribution of haptoglobin types in Chinese[19,22]. These two examples of discrepancy between blood group frequencies and the distribution of haptoglobin types show the importance of the latter in anthropological studies.

This investigation is supported by a grant from the Scientific and Technical Research Council of Turkey.

Sakir Erdem
Muzaffer Aksoy
Arif Ismet Çetingil

Çapa Internal Clinic of the Istanbul Medical School,
Vakif Guraba Hospital,
Istanbul, Turkey.

[1] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[2] Smithies, O., *Biochem. J.*, **71**, 585 (1959).
[3] Chernoff, A. I., and Pettit, N. M., jun., *J. Lab. Clin. Med.*, **63**, 290 (1964).
[4] Walter, H., Arndt-Hanser, A., Bernhard, W., and Heyde, G., *Blut*, **10**, 225 (1964).
[5] Fleischer, E., and Lundevall, J., *Fourth European Cong. Haematol., Kopenhagen*, 936 (1958).
[6] Galatius-Jensen, F., *The Haptoglobins: A Genetical Study* (Kopenhagen, 1960).
[7] Bütler, R., Rosin, S., and Walther, M., *Schweiz. Med. Wschr.*, **89**, 1041 (1960).
[8] Baitsch, H., and Liebrich, K. G., *Blut*, **7**, 27 (1961).
[9] Nyman, M., *Clin. Chim. Acta*, **3**, 111 (1958).
[10] Mäkelä, A., Eriksson, A. W., and Lehtovaara, R., *Acta Genet.*, **9**, 149 (1959).
[11] Walter, H., and Palsson, J., *Vox Sang.*, **7**, 732 (1962).
[12] Moullec, J., and Fine, J. M., *Nature*, **184**, 196 (1959).
[13] Allison, A. C., Blumberg, B. S., and Aprees, W., *Nature*, **181**, 824 (1958).
[14] Sutton, H. E., Neel, J. V., Livingston, F. B., Binson, G., Kunstadter, P., and Trombley, L. E., *Ann. Hum. Genet.*, **23**, 175 (1959).
[15] Parker, W. C., and Bearn, A. G., *Ann. Hum. Genet.*, **25**, 227 (1961).
[16] Blackwell, R. Q., and Thephusdin, C., *Nature*, **197**, 503 (1963).
[17] Kirk, R. L., Lai, L. Y. C., Mahmood, S., and Singh, R. B., *Nature*, **185**, 185 (1960).
[18] Matsunaga, E., and Murai, K., *Nature*, **186**, 320 (1960).
[19] Blackwell, R. Q., Lin, T. Y., and Shiao, D. D. V., *Res. Rep. MR* 005.09–1601.7.1 (U.S. Naval Med. Res. Unit No. 2).
[20] Aksoy, M., Ikin, E. W., Mourant, A. E., and Lehmann, H., *Brit. Med. J.*, **2**, 937 (1958).
[21] Weiner, J. S., and Zoutendyk, A., *Nature*, **183**, 843 (1959).
[22] Barnicot, N. A., Garlick, J. P., Singer, R., and Weiner, J. S., *Nature*, **184**, 2042 (1959).

Microsomal Incorporation of *N*-Acetyl-D-galactosamine into Blood Group Substance

Soluble blood group A and H substances from hog gastric mucosa consist of a protein 'backbone' to which numerous oligosaccharide units are attached[1,2]. These oligosaccharide units are supposed to consist of a chain of D-galactose and *N*-acetyl-D-hexosamines to which L-fucose residues are laterally linked[3–5]. Group A substance is considered to differ from group H substance only by an additional α-*N*-acetylgalactosamine residue at the non-reducing end of the sugar chains. In fact, substances showing H specificity can be obtained from A substances by treatment with α-*N*-acetylgalactosaminidases[6,7].

The mechanism of the biosynthesis of blood group substances is unknown. *In vivo* labelling experiments in which [^{14}C]-glycerol was injected into the gastric artery of hogs indicated that blood group substances are synthesized on the microsomes of the gastric mucosa[8]. This communication describes the transfer of ^{14}C-labelled *N*-acetylgalactosamine from uridine diphosphate (UDP) [^{14}C]-*N*-acetylgalactosamine to terminal positions of blood group H substance in the presence of microsomes from hog gastric mucosa.

A mixture of UDP-[^{14}C]-*N*-acetylglucosamine and UDP-[^{14}C]-*N*-acetylgalactosamine was prepared by injection of glucosamine-1-^{14}C (10·0 mc./m mole, obtained from New England Nuclear Corporation) into the tail vein of rats, and the UDP-*N*-acetylhexosamines isolated from the livers according to the method of O'Brien and Neufeld[9].

Microsomes were prepared from hog stomachs freshly obtained from the slaughter-house. The mucosal linings were stripped off, separated from the underlying muscularis mucosa, and homogenized in a Waring blender in 0·35 M sucrose, 0·025 M potassium chloride, 0·01 M magnesium chloride, 0·001 M ethylenediamine tetraacetic acid, 0·05 M *tris* *p*H 7·6 (3 ml. per g of mucosa). The homogenates were centrifuged at 15,000*g* for 20 min and the supernatants assayed for blood group activity by haemagglutination inhibition tests[1] and further centrifuged at 105,000*g* for 1 h. The microsomal pellet from 5 g mucosa was suspended in 1 ml. high-speed supernatant. To the microsomal suspension were added 5 μmoles adenosine triphosphate, 5 μmoles nicotinamide-adenine dinucleotide, and 0·5 μmoles UDP-[^{14}C]-*N*-acetylhexosamine (500,000 c.p.m.); the resulting mixture was then incubated at *p*H 7·6 and 37° C. In some experiments 10 mg blood group H substance (obtained from hog gastric mucosa by the procedure of Bendich *et al.*[10]) was added to this standard incubation mixture.

The incubation was terminated by cooling the incubation mixture in an ice-water bath. Then the mixture was sonicated for 5 min and, after dilution with distilled water, centrifuged at 105,000*g* for 1 h. The pellet was discarded and the supernatant dialysed against several changes of distilled water for 24 h. From the non-diffusible material the blood group substances were isolated by precipitation with three volumes of ethanol and dissolution of the precipitate in 90 per cent phenol. 50 mg blood group H substance was then added and the blood group substances re-precipitated with ethanol by adjustment of the ethanol concentration to 10 per cent by volume. The precipitate thus obtained was dissolved in water and assayed in a Packard liquid scintillation counter.

The incorporation of radioactivity from UDP-[^{14}C]-*N*-acetylhexosamines into blood group substance is shown in Table 1. With microsomes obtained from a homogenate showing blood group A activity there was a steady incorporation in the course of 2 h of incubation. On the other hand, microsomes of a blood group H-active mucosa were unable to incorporate radioactivity to any significant extent. In both experiments no labelled UDP-oligosaccharides could be detected in the diffusible portions of the incubation mixtures.

©1966 Nature Publishing Group