Results of further tests with these materials are summarized in Table II. Since the PHP was only approximately 50% soluble under the conditions used, it was necessary to conduct quantitative assays with the soluble portion only. However, the proteolytic activity of the soluble and insoluble portions of this preparation per mg nitrogen were essentially the same. The indicated amounts of soluble PHP were mixed, in duplicate, with 1 ml of 2% NGG or 1 ml of 2% IGG in a total volume of 3 ml. All solutions were prepared in $M/10$ phosphate buffer at pH 7·8, centrifuged at high speed, and filtered to remove insoluble material. The PHP-globulin preparations were incubated 30 min at 37°C and then kept at 4°C for 24 h. At the end of this time all tubes were centrifuged at 2–4°C and the supernatants saved for enzymatic analysis and checks for antigen (An) or antibody (Ab) excess by the ring test. The specific precipitates were washed three times with cold 0·15 M NaCl and total nitrogens determined (micro-Kjeldahl). (No precipitate was obtained with the NGG and hence no values on this are included in Table II.) Proteolytic activity assays were carried out on the supernatants and on a solution containing corresponding amounts of soluble PHP only.

Table II
Effect of normal and specific immune rabbit gamma-globulins on Human plasminogen*

µg Plasminogen N	Proteolytic Activity (Opt. Density)			Antigen (An)-Antibody (Ab) analyses	
	Plasminogen only	Supernatant			
		Plasminogen + NGG	Plasminogen + IGG	µg N in An-Ab ppt.	Supernatant Composition
5·3	0·040	0·040	0·002	139	Ab
10·6	0·080	0·085	0·035	241	Ab, An
15·9	0·200	0·210	0·050	296	Ab, An
26·5	0·330	0·335	0·110	352	Ab, An
42·4	0·560	0·570	0·270	360	An

* Conditions of assay given in text.

The data (Table II) indicate that the presence of IGG reduced the proteolytic activity of the supernatants markedly, while NGG had no effect. The portion of the plasminogen preparation responsible for proteolytic activity could be virtually completely precipitated in the region of large Ab excess. The results further suggested that, since both An and Ab were present in the supernatants over a broad zone, PHP probably contains more than one antigenic component. This was confirmed by using the OUDIN agar-diffusion technique[10] which demonstrated that the soluble PHP contained at least 4 antigenic components reacting with IGG. When three whole normal human sera were checked individually against IGG, multiple rings were also formed.

It was of interest to determine if IGG affected human blood clot lysis. One ml of 2% IGG in saline was mixed with 1 ml whole fresh human blood and allowed to clot. Streptokinase (1000 units) was injected into the center of these clots, as well as into two sets of control clots, one containing only saline and blood and the other 2% NGG and blood. Clot lysis was virtually complete after 90 min in the control tubes, while in those containing IGG very little lysis had taken place after 24 h. Although this is an extremely crude procedure it did furnish evidence for the inhibition of fibrinolysis in whole blood by IGG.

[10] J. OUDIN, Meth. med. Res. *5*, 335 (1952).

Thus the KLINE procedures for preparation of PHP yielded immunologically heterogeneous preparations; however, a specific antiserum was obtained which inhibited the proteolytic and fibrinolytic activity of human plasmin and formed specific precipitates. This heterogeneity makes further immunochemical studies on this plasminogen preparation of doubtful significance and renders an immunochemical assay for plasminogen impossible at this time.

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Résumé

Les auteurs ont préparé, chez le lapin, les anticorps specifiques inhibiteurs des activités fibrinolytiques et protéolytiques du plasminogène humain activé par la streptokinase. Le plasminogène humain purifié est un antigène hétérogène.

Cholesterol Blood Levels in Rh Sensitized Women Treated with Rh Hapten

In this laboratory we are engaged in an attempt to assess the value of oral administration to the Rh negative sensitized woman of the Rh hapten (a red cell fraction described previously by CARTER[1] and CARTER *et al.*[2]). Although several workers, including HOWE and RUSTIGIAN[3] and OSBORN[4], have reported inability to confirm *in vitro* observations on the Rh hapten, this inability due, it would seem, to technical variations, BARNARD[5] and GOLDSMITH[6] independently have successfully confirmed this phase of the work. WOLF *et al.*[7] stated: 'A number of our observations suggest that the term Rh hapten may correctly be applied to the extract described by CARTER'. Although WOLF *et al.*[7], MARSTERS *et al.*[8], and SPURLING *et al.*[9] have been unsuccessful in the clinical use of Rh hapten to prevent erythroblastosis, these failures due to use of too little of the fraction too late in pregnancy, EHRENBERG[10] and SCHUBERT and GRUNBERG[11] have reported clinical successes.

[1] B. B. CARTER, Amer. J. clin. Path. *17*, 646 (1947); J. Immunol. *61*, 79 (1949); Amer. J. clin. Path. *21*, 561 (1951); Lancet *1954*, 1267.

[2] B. B. CARTER, A. C. WILLIAMSON, J. LOUGHREY, and C. H. INGRAM, Jr., Amer. J. Obstet. Gynec. *72*, 655 (1956).

[3] C. HOWE and R. RUSTIGIAN, J. Immunol. *64*, 505 (1950).

[4] D. A. OSBORN, J. clin. Path. *4*, 470 (1951).

[5] R. D. BARNARD, *Proceedings of the Vth International Congress on Blood Transfusion*, p. 88 (1955).

[6] J. W. GOLDSMITH, Amer. J. Obstet. Gynec. *59*, 172 (1950).

[7] A. M. WOLF, C. SCHLUTZ, M. FREUNDLICH, and S. O. LEVINSON, J. Amer. med. Ass. *144*, 88 (1950).

[8] R. W. MARSTERS, R. T. F. SCHMIDT, and M. E. BLACK, Amer. J. Obstet. Gynec. *63*, 549 (1952).

[9] C. L. SPURLING, M. S. SACKS, and E. F. JAHN, Blood *5*, 1125 (1950).

[10] C. J. EHRENBERG, J. Lancet *75*, 275 (1955).

[11] J. SCHUBERT and A. GRUNBERG, Pediatricke listy *5*, 1 (1950).

Table I
Cholesterol Blood Levels, mg/100 ml Blood

Patient	T.P.*	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8
A	×	466	335	423	232	249	209	348	
B	×	288	213	262	177	199	265	209	
C	×	217	224	191	299	244	226	315	214
D	×	199	171	199	174				
E	×	137	216	156	173	166	171	179	
F	×	216							
G	×	166	194	181					
H	×	178							

* T.P. refers to treated, pregnant women.

Table II
Cholesterol Blood Levels, mg/100 ml Blood

Patient	T.N.P.*	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8
A	×	288	214	184	415	194			
B	×	294	166	216	173	209			
C	×	183	204	177	257	222	178	169	249
D	×	156	166	163	166	179			

* T.N.P. refers to treated, non-pregnant women.

The Rh hapten, produced as described elsewhere[2] and made convenient to handle by mixing with powdered lactose, is taken up into gelatin capsules in aliquots of not less than 100 mg. It is being supplied at present only to Rh negative, sensitized women who have lost one or more demonstrably erythroblastotic babies and who have homozygous husbands, but who are not yet pregnant again. The physician provides treatment until the Rh antibody titer is at zero when he recommends pregnancy. The hapten is supplied throughout pregnancy. Following this regimen, with patients possessing the criteria described above, in a steadily enlarging series (which will be reported elsewhere) there has been not one infant lost over a three year period. A paper describing the antibody response to oral administration is in press in the American Journal of Obstetrics and Gynecology.

In the process of investigating the effects of oral administration, we have been curious about the effect of the hapten on blood cholesterol levels. The crude fraction in use at present contains more than 35% cholesterol and/or other sterols. Modern medical interest has centered on the relation of cholesterol to atherosclerosis. Since dosage of the Rh hapten by mouth has not exceeded 200 mg daily, over eight to twelve months' periods, it was not supposed that this small quantity of exogenous cholesterol *per se* would in any way affect blood levels. However, we considered it at least possible that the administration of the Rh hapten might stimulate the production of endogenous cholesterol.

CHERNICK *et al.*[12] have demonstrated that cholesterol can be synthesized in the arterial wall. Hypercholesterolemia appears in many cases to be concomitant with atherosclerosis. The causal relationship is not clear. Nevertheless, MORETON[13] has shown that coarsely suspended substances of many kinds, as well as large fatty particles in lipemia, are removed from the blood and retained in the intima by the reticuloendothelial cells. Hypothetically, according to DEUEL[14], the protein in the arterial lining in atherosclerosis is altered from cholesterophobic to cholesterophilic substances. Presumably, union of Rh antibody with hapten could provide suspended material in the blood which might be retained in the intima and favor cholesterol deposition and/or cholesterol synthesis.

We made tests for serum cholesterol levels on each blood received from sensitized Rh negative women being given Rh hapten by mouth for a total of 61 individual bloods. This group of bloods included 38 specimens from pregnant, treated women and 23 from non-pregnant treated women. The study extended over a six months' period, during which time these women received from one to two 100 mg capsules Rh hapten daily. Bleedings were taken from these women biweekly for study of both Rh antibody titers and serum cholesterol levels.

Table III
Cholesterol Blood Levels, mg/100 ml Blood

Donor		P.N.T.	Test
	A	×	274
	B	×	332
	C	×	194
	D	×	171
	E	×	873
	F	×	236
P.N.T. refers to pregnant, non-	G	N.P.	262
treated women	H	×	294
N.P. refers to non-pregnant	I	×	184
women	J	×	171
	K	Normal	144
Normal refers to normal males	L	×	156
	M	×	163
	N	×	179
	O	×	154
	P	×	185
	Q	×	136

For controls we made tests for serum cholesterol levels on 17 bloods from individuals neither receiving nor having received Rh hapten in any form. This group included four bloods from non-pregnant women, six bloods from preg-

[12] S. CHERNICK, P. A. SRERE, and I. L. CHAIKOFF, J. biol. Chem. *179*, 113 (1949).
[13] J. R. MORETON, Science *107*, 371 (1948).
[14] H. J. DEUEL, *The Lipids*, vol. 2 (Interscience, New York 1955).

nant, untreated women and seven bloods from normal males. This seemed a sufficient number of control subjects since the normal range in cholesterol levels is firmly established and we sought only to check our own methods.

The method used in testing for serum cholesterol blood levels was that described by SCHOENHEIMER and SPERRY[15]. The proteins are precipitated from the serum by an acetone-alcohol mixture and simultaneously the cholesterol and cholesterol esters are extracted. Digitonin is used to precipitate the cholesterol after saponification and this is tested as to color development with acetic anhydride-sulfuric acid reagent, in comparison with color produced in a standard cholesterol solution. Color readings were made with a Bausch and Lomb spectrophotometer. HAWK, OSER, and SUMMERSON[16] give a range of 150 to 300 mg cholesterol in 100 ml blood as normal.

As will be seen from Table I, only five tests made on bloods from treated, pregnant women exceeded the normal range. This is of interest since DEUEL[14] states that cholesterol levels are normally higher in pregnancy. Four of these tests are on bloods from the same woman, drawn at different times. In Table II, which deals with treated non-pregnant women, there is one specimen of blood in which the normal cholesterol range is exceeded. Among the pregnant, non-treated group (Table III) there are two bloods which exceed normal levels. One of these findings, at a level of 873 mg cholesterol, was confirmed in another laboratory. None of the normal bloods exceed the range for cholesterol.

Apparently the oral administration of Rh hapten does not affect serum cholesterol levels. Instead, there was a certain amount of fluctuation from one test to another on the same individual, nearly all of these within the normal range.

In summary, 61 bloods from Rh sensitized women treated with Rh hapten given orally were tested for serum cholesterol levels, using 17 control bloods from pregnant, non-treated women, non-pregnant women and normal males. Differences in cholesterol levels between the treated and control groups were not significant.

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Zusammenfassung

Cholesterin lässt sich als Bestandteil des ungereinigten Rh-Haptens nachweisen. Deshalb wurde geprüft, ob die perorale Aufnahme dieser Fraktion den Blutcholesterinspiegel beeinflusst. Bei 17 Menschen, die kein Hapten erhielten, und bei 61 Hapten-Empfangenden wurde keine Erhöhung des Cholesterinspiegels festgestellt.

[15] R. SCHOENHEIMER and W. M. SPERRY, J. biol. Chem. *106*, 745 (1934).

[16] P. B. HAWK, B. L. OSER, and W. H. SUMMERSON, *Practical Physiological Chemistry* (Blakiston, New York 1954).

Influence of Blood Sugar Level on the Glycolytic Activity of Human Red Cells

Considerable evidence has been collected in the last few years on the importance of glucose as the main source of energy for the red cells[1]. Mature erythrocytes utilize glucose mainly by means of glycolysis, i.e. by anaerobic fermentation to pyruvic and lactic acids, only a very small fraction being oxydized aerobically. The hexose-monophosphate shunt has been recently demonstrated to occur also in the red cells and appears to be an important metabolic pathway[2]. Abnormalities of the glycolysis and related enzymes have been described in the *in vitro* storage as well as in a number of hemolytic conditions and are likely to occur also during the *in vivo* ageing.

But the ultimate significance of glycolysis deserves further study. Among the many problems which have still to be answered, an important point is the following: do red cells utilize a fixed amount of glucose for their metabolic requirements regardless of external factors? Or do they have to be considered as simple enzyme parcels passively reacting in a peripheral medium and metabolizing any suitable substrate? Or might their metabolic activity be influenced by the environmental conditions and adjust itself to plasma composition?

The statement generally reported, that the glycolysis rate does not depend upon the initial glucose level within normal ranges, would support the first possibility aforementioned. In the present paper some contradictory results are reported.

Materials and methods.—The investigations have been carried out in normal individuals ranging from 22 to 35 years of age. Glycolysis has been determined according to HOLLINGSWORTH's technique[3]. Blood sugar was measured in duplicate before incubation, and after 1 and 2 h, by means of the SOMOGY-NELSON's method. The standard error for the determination of glucose in duplicate was $\pm$ 2·7 mg%.

Results.—The samples were withdrawn 1–4 h after lunch. In the reconstituted samples, the red cell count ranged from 3 360 000 to 5 200 000 and the hemoglobin from 9·9 to 14·9 g%. The white cells were found between 0 and 800 per mm^3. The RBC: WC ratio ranged from 4000 to 50 000. No changes of the hematocrit were observed at the end of the experiments.

The mean initial glucose level was 76·2 mg% with a standard deviation of $\pm$ 17·9. It dropped to 40·2 mg% $\pm$ 11·4 after 2 h. Glycolysis, expressed as mg of glucose utilized by 100 ml of packed red cells per hour, has been found to be 43·9 mg with a standard deviation of $\pm$ 13·6. The percentage in respect to the initial glucose level was 23·7 $\pm$ 4·6.

By plotting, on a normal graphic, the glycolysis values against the initial glucose level, a fairly good correlation was observed and confirmed statistically. In our 40 normal controls, the correlation coefficient (r) was found to be + 0·742 and the calculated 't' value (6·822) was highly significative ($P < 0{\cdot}001$; Figure). The regression line is given by the formula: $y = 7{\cdot}4 + 0{\cdot}48\,x$, where y is the expected glycolysis and x the initial glucose level. The confidence limits ($2\,\sigma$) are $\pm$ 15.

According to these data, glycolysis could be tentatively interpreted as a first order enzymatic reaction. Since, in this case, the amount of substrate (S) present at the time t is given by the formula: $S_t = S_0\, e^{-kt}$, k being the velocity constant, the reaction itself could be better expressed

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[2] O. F. DENSTEDT, in J. L. TULLIS, *Blood cells and plasma proteins* (Academic Press Inc., New York 1953), p. 223.

[3] J. W. HOLLINGSWORTH, J. Lab. clin. Med. *45*, 920 (1955).