1. In every case a blood sugar increasing and a blood sugar decreasing principle was clearly demonstrated.

2. The type of curve obtained was dependent upon the varying amounts of either substance present.

3. Predominance of the toxic fraction (blood sugar increasing substance) invariably produced a preliminary rise in the blood sugar which was followed by a fall below normal. The period of delay in the fall of blood sugar varied with the amount of the toxic substance present.

4. By methods of purification, which will be reported later, it is possible to separate to a very large degree the hypoglycemia-producing principle from the hyperglycemia-producing substance; at least by these methods the double effect described when both substances are present, can be eliminated and a continuous rise or fall of the blood sugar can be obtained.

5. The hypoglycemia-producing principle and the hyperglycemic substance are present in the juice, pulp and rind of the fruits we have examined.

6. The hypoglycemia-producing principle acts similar to insulin. No delayed action has been observed as described for "Glucokinin".

The above findings appear to be in agreement with those reported by Dubin and Corbitt for vegetable extracts. At present it is impossible to state whether the slightly delayed fall in blood sugar of normal rabbits is a qualitative or a quantitative phenomenon of the toxic fraction.

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The nature of human isohemagglutinogens.

By L. G. HADJOPOULOS and REGINALD BURBANK (by invitation).

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In a previous communication on the nature of specific hemolysins, one of us[1] has dealt on the complexity of erythrocytes as antigen and endeavored to demonstrate that the cell stroma is the

[1] Hadjopoulos, L. G., *Arch. Int. Med.*, 1921, xxvii, 441.

main, if not the sole source of hemolysinogens. In this paper, as a supplementary study, we have undertaken to demonstrate that isohemagglutinogens, in a similar way, originate in the cell stromata.

To obtain cell stromata in a more or less pure condition and individually from each of the four different types of bloods we used the same technique as discussed in our previous communication. In short it consists of: *First,* isolating the four types of erythrocytes. Samples of blood, left over from Wassermann reactions were used for this purpose. *Second,* filtering through gauze and centrifugalizing the saline suspension of broken clots. This step was repeated until the supernatant fluid was free of serum ingredients. *Third,* hemolyzing the final sediment in 0.45 per cent saline solution and sedimenting stromata by centrifugalization. To get rid of hemoglobin further sedimentations were done in physiological saline to avoid deterioration of cell stromata.

The final product, when examined under the microscope, should show the cell stromata intact, more or less globular, varying in size from that of a blood platelet to half the size of an erythrocyte and devoid of hemoglobin. Presence of hemoglobin means deficient "dehemoglobinization", which will necessitate repetition of step three until dehemoglobinized cells (cell stromata) look almost transparent.

TECHNIQUE

The four types of serums are isolated, washed free of their cellular constituents and divided in amounts of 0.20 cc. into four small tubes. This gives sixteen tubes, arranged in four rows and of four tubes each. The first row of tubes, representing the four types of serums, receives a sufficient amount of type I cell stromata, the second row, type II cell stromata, etc. The cross absorption test is then carried out in the incubator for 30 minutes and over night in ice box. The following morning all tubes are centrifugalized and the clear serums transferred in the same order to another set of sixteen tubes. The first row now represents type I absorbed serum, the second type II, etc.

A preliminary test of the degree of absorption is done by taking a drop of serum from tube No. 3 second row, a drop from tube No. 2 third row and one from tube No. 1 fourth row. After

placing these drops in their order on a clean slide a drop of group III cell suspension is mixed with drop No. 1, group II cells with drop No. 2 and group I cells with drop No. 3. The slide is now kept at room temperature for 30 minutes under a petri dish cover to prevent drying. If no agglutination occurs at the end of this time it is safe to consider the absorption as complete.

The original sixteen tubes are now centrifugalized at high speed for ten minutes and then arranged in their original order. Eight clean and dry glass slides are set in succeeding order with a cover for each. The first slide gets four drops from tube No. 1 and under these another four drops from tube No. 2. In a similar way the other seven slides are prepared and covered. Of the four rows of drops, with sixteen in each row, No. 1 gets a drop of type I cell suspension, No. 2 a drop of type II cells, etc. At the end of 30 to 40 minutes the results are read macroscopically and, if need be, also microscopically. Table I demonstrates our results.

TABLE I.

Results of cross absorption tests with cell stromata.

Type serums absorbed with type stromata	Unabsorbed agglutinin after absorption	Washed, intact erythrocytes							
		i (AB)		ii (A)		iii (B)		iv (0)	
		Theor.	Act.	Theor.	Act.	Theor.	Act.	Theor.	Act.
I - i	0 - AB = 0	—	—	—	—	—	—	—	—
I - ii	0 - A = 0	—	—	—	—	—	—	—	—
I - iii	0 - B = 0	—	—	—	—	—	—	—	—
I - iv	0 - 0 = 0	—	—	—	—	—	—	—	—
II - i	b - AB = 0	—	—	—	—	—	—	—	—
II - ii	b - A = b	+	+	—	—	+	+	—	—
II - iii	b - B = 0	—	—	—	—	—	—	—	—
II - iv	b - 0 = b	+	+	—	—	+	+	—	—
III - i	a - AB = 0	—	—	—	—	—	—	—	—
III - ii	a - A = 0	—	—	—	—	—	—	—	—
III - iii	a - B = a	+	+	+	+	—	—	—	—
III - iv	a - 0 = a	+	+	+	+	—	—	—	—
IV - i	ab - AB = 0	—	—	—	—	—	—	—	—
IV - ii	ab - A = b	+	+	—	—	+	+	—	—
IV - iii	ab - B = a	+	+	+	+	—	—	—	—
IV - iv	ab - 0 = ab	+	+	+	+	+	+	—	—

Note. Under the first column the capital Latin numerals stand for the various types of serums; small numerals for the various group stromata used to absorb the corresponding serums. In the second column, the small letters stand for the agglutinins and the capitals for the agglutinogens. In the other four columns the small Latin numerals represent the type of the intact erythrocytes. The sign of plus means agglutination; the negative sign absence of agglutination. Under each column there are two readings: the first is what we should get theoretically and the second what we actually got in the course of our experiments.

The close parallelism of the theoretical and actual findings leaves no doubt in our opinion that "dehemoglobinized" cells (cell stromata) are the main, if not the only source of hemagglutinogens.

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Serological examination of a species-hybrid.

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Although of some general interest, the question of the heredity of species-specific properties in animals as indicated by serum reactions has not yet been investigated. While some difficulty exists in discriminating substances derived from closely related species by precipitins, it is easy to do so by the use of hemagglutinins.

After producing agglutinins in rabbits with the blood, for instance of the horse or donkey, one obtains, by absorption with the heterologous blood, solutions which act specifically on the blood of the species used for immunization. In this way the blood corpuscles of a species-hybrid, namely the mule, were examined by means of immune sera against horse, donkey, and mule blood. Of the several possibilities, such as conformity of the blood of the hybrid with that of one parent, a combination of the properties of both, or formation of new substances, the one which actually occurs is the inheritance of agglutinable substances of each parent.

It remains to be estabilished whether the results are different in the cross between the mare and the jack and the reciprocal cross.

We hope to extend the studies to cases of fertile hybrids.