scribed for isolation of human thrombin from plasma Fraction III in 40-50% yield. Average specific activities of these preparations are 10,300 "Iowa" units per mg protein, 69,000 units per mg nitrogen, and 99,400 units per mg tyrosine. The higher activity of human thrombin indicates that it is either a smaller molecule or more active than bovine or equine thrombin.

1. Lamy, F., Waugh, D. F., *Physiol. Rev.,* 1954, v34, 722.

2. Rasmussen, P., *Biochim. Biophys. Acta,* 1954, v16, 157.

3. Seegers, W. H., Levine, W. G., Shepard, R. S., *Can. J. Biochem. Physiol.,* 1958, v36, 603.

4. Magnusson, S., *Acta Chem. Scand.,* 1958, v12, 355.

5. Gladner, J. A., Laki, K., *J. Am. Chem. Soc.,* 1958, v80, 1263.

6. Seegers, W. H., Casillas, G., Shepard, R. S., Thomas, W. R., Halick, P., *Can. J. Biochem. Physiol.,* 1959, v37, 775.

7. Miller, K. D., *Nature,* 1959, v184, 450.

8. Seegers, W. H., Landaburu, R. H., *Canad. J. Biochem. Physiol.,* 1960, v38, 1405.

9. Cohly, M. A., Scheraga, H. A., *Arch. Biochem. Biophys.,* 1961, v95, 428.

10. Ware, A. G., Seegers, W. H., *Am. J. Clin. Path.,* 1949, v19, 471.

11. Lewis, M. L., Ware, A. G., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 636.

12. Steichele, D. E., Mammen, E. F., Hatta, Y., Seegers, W. H., *Thromb. Diath. Haem.,* 1960, v4, 365.

13. Miller, K. D., McGarrahan, J., *Annual Report of Division of Laboratories and Research,* N. Y. State Dept. Health, Albany, 1958, p69.

Received September 14, 1962. P.S.E.B.M., 1962, v111.

In vitro Cell Migration as a Model for Delayed Hypersensitivity.* (27841)

MARIAM GEORGE AND JOHN H. VAUGHAN (Introduced by W. F. Bale)
Department of Microbiology and Medicine, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

One of the most pressing problems in the study of delayed hypersensitivity is whether there is an adequate *in vitro* model of this state of altered reactivity. Rich and Lewis (1) reported that explants of spleens and lymph nodes from tuberculin sensitive animals grow out poorly in the presence of tuberculin, but do quite well in its absence. Similar observations have been made(2) on the outgrowth of cells of animals with delayed hypersensitivity to other bacteria. Inhibition was not seen with cells from animals anaphylactically sensitive to soluble antigens(3). Chase(4) noted that tuberculin hypersensitivity, though not transferred with serum, could be transferred with spleen or lymph node cells. Miller and Favour(5) attempted to provide a simple *in vitro* model of tuberculin sensitivity with a system of peripheral blood or splenic sensitive leukocytes, serum, and complement, in which lysis of cells was noted when the cells were made to tumble for relatively short times in a rotating drum at 37°C. Evidence accrued(6), however, that Favour's "cytolytic" effect could be seen with normal cells in the presence of added antigen, antibody, and complement, and doubt has consequently been expressed that the model really represents delayed sensitivity.

Interest in macrophages as cells implicated in delayed hypersensitivity has gradually developed. Rich(7) pointed to the disposition for macrophages to collect in the lesions of tuberculous animals, and commented upon the ability of the wax of the tubercle bacilli to evoke this type of cellular response. Injection of tuberculoprotein together with tuberculowax has been reported by Raffel(8) to result in development of delayed hypersensitivity to the protein, while immunization with tuberculoprotein alone evoked only anaphylactic hypersensitivity. Gangarosa *et al.*(9) pointed out that addition of antigen to spleen cultures from animals with delayed sensitivity to tuberculin or to mumps virus had a demon-

* Supported by grant from Nat. Inst. Health, U.S.P.H.S.

strable effect on outward migration of macrophages, but not of lymphocytes. Waksman and Matoltsky(10) have implicated macrophages both in tissue culture and in transfer experiments.

The present studies were constructed to provide further information on the possibility of using macrophages in an *in vitro* model for delayed hypersensitivity. The effect of specific antigen on the migration of peritoneal exudate cells, spleen cells, or lymph node cells from sensitized animals has been tested. For comparative purposes, the migration studies have been accompanied by tests for serum antibody in the animals donating the cells and by assessment of their skin test reactivities.

Materials and methods. Immunization. Two means of immunization have been employed. White male guinea pigs (300-400 g) were injected subcutaneously with 4 mg of live BCG† in saline. After about 3 months, the animals were used for skin testing or to provide cells for tissue culture. Some of the animals were injected at this time with 2-5 mg of egg albumin intravenously or intramuscularly on each of 4 occasions at intervals of 3 to 4 days; these animals were sacrificed for study 5 days after the last egg albumin injection.

Another group of animals were immunized with egg albumin in Freund's adjuvant. Two dml of egg albumin (250 μg Ea N/ml) was homogenized in 7:5 ml of mineral oil, 4.5 of aracel A and 8 mg BCG. Animals were injected with 0.1 ml of the mixture in each paw.

Collection of cells. Four to 5 days before an experiment, 25-30 ml of mineral oil was injected intraperitoneally to induce exudates. Three to 4 days later half the animals were skin tested with 10 μg of PPD or egg albumin nitrogen in 0.1 ml. After observing the skin reaction for 24 to 48 hours, both skin tested and non-skin tested animals were bled from the heart and sacrificed by a blow to the head. The peritoneal exudate cells were collected by washing them out with 200 ml of balanced salt solution. The spleens and lymph nodes were removed and cells collected from them by forcing the diced tissues through a Baxter stainless steel 60-mesh blood transfusion filter. Alternatively, the organs were teased carefully, releasing the cells into balanced salt solution and the gross tissue fragments separated by pouring the suspension through the stainless steel sieve. The cells were washed 3 times, an aliquot counted in a hemocytometer, and the remainder suspended in a volume of nutrient medium sufficient to provide the desired cell density.

Passive transfer (cf. *6, 18*). 1×10^7 cells were mixed with varying concentrations of PPD‡ in total volumes of 0.1 ml and immediately injected intradermally into 350-400 g normal white male guinea pigs. The consequent skin reactions were measured at 24 and 48 hours. Two diameters at right angles to each other were measured by a centimeter ruler. The skin thickness at areas of induration and at adjacent normal skin areas was measured with slide calipers. The volume of the skin reaction was calculated by multiplying the 2 measured diameters by the calculated thickness of the lesion and by $\pi/4$ (10).

Migration studies. 1.5-1.8 $\times$ 10^7/ml of cells were mixed with varying concentrations of PPD in modified Eagle's medium containing 15% normal homologous serum. Capillary tubes of 7.5 by 0.07 cm were filled in replicate with these cell suspensions. One end of the tube was closed with soft paraffin. The tubes were then centrifuged at 500-600 rpm for 5 minutes, after which they were broken off at the interface between the packed cells and the supernatant. The broken ends containing the cells were placed in Mackaness chambers(11) on the bottom coverslip and held in place by means of a small spot of silicone lubricant. The silicone was found to be harmless for the cells. The top coverslip of the chamber was fixed in place with paraffin and the chambers filled with about 1 ml of nutrient medium containing PPD in the amount used in the original suspending fluid. The chambers were incubated for 24 and 48

† The BCG preparation used was a lyophilized vaccine obtained from the Research Foundation, Chicago, Ill.

‡ PPD (Lot No. 95923) was provided by courtesy of Dr. J. R. Robinson, Merck Inst. for Therapeutic Research, Rahway, N. J.

TABLE I. Reaction Volumes* at 24 Hours in the Skins of Normal Guinea Pigs Injected with PPD Mixed with Peritoneal Exudate or Spleen Cells from BCG Sensitized and Non-sensitized Animals.

	Sensitized donor animals								Non-sensitized donor animals			
PPD conc.,	Non-skin tested				Skin tested†							
μg/ml	1	2	3	4	1	2	3	4	1	2	3	4
Peritoneal exudate cells												
0	11.2	10.8	5.1	2.4	10.9	.5	0		1.6	0	0	2.5
7.5	73.2	34.1	29.6	98.2	20.8	27.6	15.0		.9	0	7.3	4.1
15	69.6	47.7	50.0	75.0	31.7	18.5	11.0		1.2	4.1	0	3.9
30	33.5	28.7	21.8	51.0	13.0	8.5	5.0		0	0	6.4	1.4
60	13.2	0	17.9	15.0	10.4	11.2	24.0		0	0	3.6	0
Spleen cells												
0		0	0		0	0				0	0	
7.5		0	8.8		0	1.35				<1	0	
15		0	6.5		0	2.7				<1	0	
30		0	5.9		0	0				0	0	
60		0	0		0	0				0	0	

* See Passive Transfer under *Materials and methods.*
† Skin tests with PPD were performed 3 days prior to harvesting cells from the donors.

hours at 37°C. The cells migrated out, fanlike, from the broken capillary onto the lower cover glass. An image of the area into which the cells had migrated was projected onto drawing paper with a Bausch and Lomb arc projector. The projected area could then be measured by a planimeter and the absolute area of migration in the chamber calculated by the known magnification factor.

Antibody formation in vitro. 3.5×10^7 washed cells were put into 10 ml beakers in duplicate in 1 ml of modified Eagle's medium. One of each duplicate was frozen and thawed twice to serve as a control. All were incubated at 37°C in 5% CO_2 and 95% oxygen for 48 hours. The cells were removed by centrifugation in the cold and the supernatants were analyzed for hemagglutinating antibody by tanned cell hemagglutination(12).

Results. In all the observations to be reported, unless otherwise indicated, cells were collected from animals on which no prior diagnostic skin testing had been performed, as it was felt that skin tests might significantly effect the *in vitro* behavior of the subsequently harvested cells. Accordingly, all experiments were done with two sets of animals identically prepared, one set for the skin testing and the other for cell donations.

When peritoneal exudate cells from guinea pigs sensitized with BCG 3 months previously were mixed with PPD and immediately injected intracutaneously into normal animals, skin reactions exhibited by erythema and induration resulted at the sites of injection at 24 hours (Table I). Spleen cells from the same animals, and cells from non-sensitized animals, failed to give significant skin reactions under the same circumstances. Cells from donors which had had skin tests 3 days previously gave lesser reactions than those from animals which had had no previous skin tests. In experiments not shown in the table, serum from the same donors, mixed with the various concentrations of PPD, failed to induce observable skin reactions.

In migration studies *in vitro* (Table II), peritoneal exudate cells from sensitized animals were strikingly inhibited in the presence of PPD. This was less conspicuous or absent with spleen cells from the same donor animals. Peritoneal exudate cells from non-sensitized guinea pigs grew out quite well in the PPD.

To compare the behavior of peritoneal exudate cells and spleen cells from BCG sensitive animals which also were anaphylactically sensitive to a different antigen, a set of BCG sensitized guinea pigs (infected with BCG 3 months previously) were immunized with alum precipitated egg albumin by injections of 2-5 mg every third day for 4 times. The first and the last injections were intravenous; the middle 2 intramuscular or subcutaneous. On the day after the last intravenous injection, half the animals were given mineral oil

TABLE II. *In vitro* Migration of Cells from BCG-Infected or Normal Animals.

Exp. No.	Time of observation (hr)	Areas (mm²) occupied by migrating cells: Spl. cells† PPD/ml 0	Spl. cells† PPD/ml 30γ	P.E. cells‡ PPD/ml 0	P.E. cells‡ PPD/ml 30γ	Migration of P.E. cells in PPD (% of control)
		Sensitive donor animals				
1	24			2.5*	<1	<43
				2.2*	<1	
	48			6.2	<1	<19
				4.6	<1	
2	24	5.2*	5.0	3.0	1.2	43
		5.0*	4.9	2.2	1.0	
	48	5.2	5.6	4.5	1.5	35
		5.6	5.4	3.6	1.3	
3	24	7.2	3.5	8.5	2.5	33
		7.4	3.3	6.3	2.3	
	48	8.5	4.2	8.8	3.5	42
		8.5	4.5	7.5	3.4	
4	24	8.8	7.2	4.0	<1	<27
		8.8	7.5	3.5	<1	
	48	8.8	8.3	8.2	2.5	31
		8.8	8.5	7.9	2.5	
5	24			2.2	<1	48
				2.0	<1	
	48			6.8	<1	<15
				6.6	<1	
6	24	7.7	5.5	2.7	<1	<37
		5.3	5.0	—	<1	
	48	8.2	7.2	5.0	1.8	<34
		7.0	6.8	—	1.6	
		Normal donor animals				
1	24			5.5	4.8	84
				5.7	4.6	
	48			—	7.2	
				—	7.0	
2	24			9.2	9.0	101
				9.0	9.5	
	48			9.5	9.2	
				9.2	8.5	
3	24			2.5	2.8	95
				2.7	2.6	
	48			5.8	5.8	95
				5.9	5.3	
4	24			3.5	4.8	140
				3.6	5.2	
	48			5.5	5.2	94
				5.7	5.3	
5	24			9.2	9.0	96
				9.0	8.5	
	48			9.5	9.2	95
				9.2	8.5	

* Duplicate determinations, single chamber.
† Spleen cells.
‡ Peritoneal exudate cells.

intraperitoneally. Peritoneal exudate cells, spleen cells, and lymph node cells were collected from them 4 days later. The other half of the animals were skin tested with 10 γ of PPD and 10 γ of egg albumin nitrogen. The characteristics of the skin tests were given in the left side of Table III. Strong early, edematous reactions against egg albumin were seen, and well-developed delayed, indurated, erythematous reactions were seen with PPD.

In *in vitro* studies, migration of the peritoneal exudate cells was inhibited by PPD, but not significantly by 2 different concentrations of egg albumin (left side of Table IV).

Spleen cell migration was not significantly affected by either Ea or PPD in these experiments. On the other hand, the spleen cells were capable of producing antibody against egg albumin *in vitro*. Equal numbers of the peritoneal exudate cells did not make antibody under identical culture conditions. The sera of all animals showed good titers of hemagglutinating antibody to egg albumin. Hemagglutination of tanned cells coated with PPD, however, was not demonstrable, save in sera from animals that had been skin tested 3 days previously (*cf.* 13).

To ascertain whether a different mode of immunization with egg albumin makes any difference in the types of skin reactions and *in vitro* migrations seen, a group of normal 350-450 g guinea pigs were injected with egg albumin in Freund's adjuvant. On the seventh and twelfth days after these injections, half of the animals were skin tested with egg albumin and PPD and the other half were sacrificed to collect the serum and the peritoneal exudate, spleen, and lymph node cells. Portions of the cells were cultured for antibody production *in vitro* and other portions were set up in chambers for migration studies. The results are shown in Tables III, IV, and V.

The skin test results (Table III) closely resemble the patterns emphasized by Gell and Benacerraf(14) and by Salvin and Smith (15). Animals skin tested 7 days after immunization showed delayed type skin reactions against both PPD and egg albumin. At 12 days, PPD still gave rise to delayed reactions with little or no early edematous phase.

TABLE III. Skin Test Responses in Animals Sensitized by Two Methods.

Skin test antigen	BCG-infected animals immunized with alum precipitated Ea and tested 5 days after last antigen inj		Animals immunized with Ea in Freund's adjuvant			
			Tested at 7 days		Tested at 12 days	
	6-hr edema	24-hr induration	3-6-hr edema	24-hr induration	3-6-hr edema	24-hr induration
	mm					
10 μg Ea N i.c.	28 × 27	—	tr	16 × 18	30 × 30	18 × 18
	28 × 28	--	—	12 × 14	22 × 22	18 × 18
	20 × 22	—	tr	15 × 16	18 × 20	12 × 15
			—	15 × 20	30 × 30	25 × 28
					30 × 30	15 × 15
					30 × 28	15 × 14
10 μg PPD i.c.					—	15 × 17
	5 × 8	18 × 19	tr	14 × 15	8 × 10	18 × 17
	—	22 × 25	tr	13 × 14	8 × 10	12 × 15
	—	18 × 18	tr	15 × 16	—	18 × 14
			—	18 × 20	—	15 × 16

However, egg albumin at this time provoked vigorous early as well as delayed responses.

In *in vitro* migration (Table IV), egg albumin significantly inhibited the migration of peritoneal exudate cells from adjuvant-injected animals. The figure of 80% was taken to represent the dividing line between significant and insignificant findings in suppression of cell outgrowth, since in previous observations non-sensitive cells had always exhibited in the presence of antigen 80% or more of the outgrowth of the control cells. Inhibition by egg albumin was best seen in experiments performed with cells taken from animals 7 days after immunization. In experiments performed at 12 days, inhibition of migration by egg albumin was seen in only half the experiments. Inhibition by PPD was seen in all experiments at 7 days and in 5 of 6 instances at 12 days.

Spleen cells of the adjuvant-sensitized animals also appeared to be inhibited in migration both by PPD and by Ea in 3 experiments performed at 7 days. The results with spleen cells generally, however, were totally irregular. This irregularity cannot be explained and casts doubt on the reliability of using spleen cells as an indicator in this study. The data, therefore, have not been included in the tables. It may be significant, however, that hemagglutinin was demonstrated irregularly and only in low titer in the sera of adjuvant-injected animals at 7 days, but regularly and in high titer at 12 days (Table V). Spleen and regional lymph node cells at 12 days made demonstrable antibody *in vitro* in 3 of 5 and in 5 of 7 instances, respectively. Peritoneal exudate cells *in vitro* made no detectable hemagglutinin in any instance.

Discussion. Since Rich and Lewis(1) re-

TABLE IV. Inhibition of 24-Hour Migration of Peritoneal Exudate Cells in Tissue Culture.

BCG-infected animals immunized with alum precipitated Ea and tested 5 days after last antigen inj			Animals immunized with Ea in Freund's adjuvant					
			Tested at 7 days			Tested at 12 days		
γ Ea N/ml		γ PPD/ml	γ Ea N/ml		γ PPD/ml	γ Ea N/ml		γ PPD/ml
10	20	30	10	20	30	10	20	30
87*		42	57		39	58	59	135
100	120	49	62		40	43	44	63
	111	55	50		20	108	109	42
99		29	83		40	122	122	45
123†		30†	52	40	59	85	88	50
						48	53	49

* Migration as % of control.

† Examined after 48 hr *in vitro*.

TABLE V. Hemagglutinin Titers in Supernatants of 48-Hour Cultures of Cells from Guinea Pigs Immunized Previously with Egg Albumin in Freund's Adjuvant.

Days after immunization	Animal No.	Animal Serum titer	Spleen cells	Peritoneal exudate cells	Regional lymph node cells
7	644	80	0	0	
	645	80			
	646	40	0	0	
	647	0			
	648	0	0	0	
	649	0	0	0	
	650	20	0	0	
	651	0	0	0	
12	866	5120		0	320
	867	>10240			40
	869	>10240	20	0	20
	870	>10240	80	0	40
	879	>10240	0	0	640
	607	160	0	0	0
	614	10240	80	0	0

ported that antigen is able specifically to inhibit migration of cells from hypersensitive animals in tissue culture, there have appeared conflicting reports of the usefulness or validity of this as an *in vitro* model of delayed hypersensitivity. Some belief has grown that the results might have been entirely factitious, depending upon some toxicity inherent in the antigens themselves, and that there is no such thing as "cytotoxicity" as an expression of delayed sensitivity. This impression probably has been derived in part from the fact that some workers have sought evidence of and found no "cytotoxicity" with cells outside of the lymphoid tissue, *e.g.*, epithelial cells(16), a finding more likely indicative that such cells are not directly involved in delayed hypersensitivity. Studies on lymphoid cells in the presence of antigen have sometimes been performed with antigens which certainly must have been so toxic (*e.g.*, extracts from *B. coli* cultures, reference 17) as to obscure any results that might be attributable to hypersensitivity itself. The demonstration that Miller and Favour's "cytolytic" breakdown of leukocytes(5), when they are tumbled in the presence of antigen, can also be brought about by classical antigen-antibody systems has emphasized that Miller and Favour's phenomenon probably did not truly reflect tuberculin hypersensitivity, but rather the presence of humoral antibody to tuberculin and interaction of this antibody with tuberculin at the cell surface.

The present report, and some further experience with conjugated proteins, has emphasized to us that significant inhibition of migration of peritoneal exudate cells in tissue culture is seen restrictively with cells from animals in which delayed type skin reactions can be demonstrated. When only immediate type skin responses occurred, no inhibition occurred.

It will be recognized that the precise specificities involved in the skin test responses and the hemagglutination reactions have not been defined. It is possible, for instance, that only impurities in the egg albumin are involved in the reactions observed. This has not seemed to us, however, to invalidate the fact that the stage, or state, of the animal's immunological responsiveness (delayed hypersensitivity *vs.* immediate hypersensitivity) to a soluble, non-bacterial antigen could be correlated with a given type of behavior of the animals' cells *in vitro*.

Both with the Metaxas technic(18) of passive transfer and with *in vitro* inhibition of migration, peritoneal exudate cells have provided better systems for inhibition than have spleen cells. A lack of correlation of *in vitro* inhibition of cell migration with level of serum antibody in the donor animal emphasizes the probability that delayed hypersensitivity and *in vitro* inhibition of cell migration are not mediated by ordinary antibody. It is noteworthy that the peritoneal exudate cells, cells very susceptible to *in vitro* inhibition of migration, failed to make demonstrable amounts of the humoral antibody in tissue culture; spleen cells, poorly inhibited in migration, made antibody in good quantity.

On these bases, support is built for the postulation that inhibition of *in vitro* migration of peritoneal exudate cells is an equivalent of delayed hypersensitivity and is a phenomenon probably different from Favour's "cytolysis." The *in vitro* model should therefore be useful as a tool for controlled studies of delayed hypersensitivity, especially for an assessment of some of the critical variables

influencing delayed hypersensitivity. It is possible that the mineral oil used in eliciting the exudative peritoneal response is such a critical item in the system. Marks(19) has presented evidence that inhibition of migration of cells by antigen requires some concomitant compromise in cell function, such as an atmosphere deficient in oxygen. It may be that ingestion of oil droplets by the exudate cells in the present study imposes upon them an increased metabolic demand that would give in essence the hypoxia that Marks described.

It is unfortunate that cytological studies so far have failed to define precisely which cells are involved in the inhibition. Since the peritoneal exudate suspension generally consists of 70% or more macrophages and the spleen cells of 70% or more lymphocytes, it seems likely that these respective cells are responsible for the gross migrations observed. Attempts to differentiate cell types after 24 or 48 hours *in vitro* were unsuccessful, as the cells in all cultures tended to round up, become pyknotic, and thereby lose their differential morphologic characteristics. The principal cell of the peritoneal exudates is referred to as a macrophage, since it is a large cell with ingested oil particles and has a large, irregular, open nucleus. It may be, as suggested by Rebuck's studies(20), that this is actually a type of lymphocyte which has differentiated into a macrophage. Under any circumstance, it is a cell that makes little or no antibody (Table V). There are no data to indicate what might be the relationship between these peritoneal exudate cells and the stromal cells of the spleen, which Eisen *et al.* (21) have noted to have a greater capacity for transfer of tuberculin sensitivity than is exhibited by splenic lymphocytes.

Waksman and Matoltsky's descriptions(10) of stimulation of macrophage proliferation *in vitro* by tuberculin are descriptions of later cellular events than those described here. It cannot be told yet whether the macrophage proliferation described by Waksman and Matoltsky is due to a primary stimulation by antigen of the macrophages to proliferate, or whether the antigen-cell interaction releases growth supporting substances similar to those obtained from cells of a feeder layer(22) in tissue culture.

Tuberculin inhibits migration of peritoneal exudate cells in tuberculin hypersensitivity. Egg albumin also inhibits, when delayed skin reactions to egg albumin exist. Raffel(23) has properly emphasized, however, that it may be prudent not to equate too hastily bacterial hypersensitivity and the delayed hypersensitivity inducible with simple proteins such as egg albumin after their injection in Freund's adjuvant. Raffel has preferred to designate the latter as Jones-Mote hypersensitivity(23), because of the more evanescent characteristic of this phenomenon, and for other reasons. The present findings nevertheless point up a characteristic to be added to those by which the two types of hypersensitivity are indistinguishable.

Although emphasis has been placed on the peritoneal exudate cells in these studies, inhibition of spleen cell migration was sometimes noted. These results, however, were highly irregular. In some of the spleen cell experiments the control cells migrated significantly less well than the test cells. It is possible that active antibody formation by the spleen cells provides a way to neutralize the antigen and protect the cells from injury by antigen (*cf.* 24). It is also possible that the observed variability of spleen cell migration is due to variable physical damage to the spleen cells incurred during sieving, a procedure not necessary in preparing peritoneal exudate cell suspensions. A third possibility is that delayed and immediate hypersensitivities are functions of primarily different cell types, *i.e.*, the macrophage on the one hand and the lymphocyte-plasma cell on the other. Experiments to clarify these uncertainties are needed.

Summary. A study has been made of the abilities of peritoneal exudate cells and spleen cells to migrate from capillary tubes in a tissue culture system. In the presence of PPD, peritoneal exudate cells from animals sensitive to PPD failed to migrate normally, while spleen cells show no consistent response. Inhibition of peritoneal exudate cells in the presence of egg albumin occurred if sensitization had been performed so that delayed skin

reactions to egg albumin existed. Inhibition did not occur when early (3-6 hr) edematous reactions to skin testing existed, when significant amounts of circulating antibody to egg albumin were demonstrable. Thus the ability of antigen to inhibit cell migration in tissue culture has been confirmed with peritoneal exudate cells for tuberculin hypersensitivity and this *in vitro* characteristic has been shown to apply also to the delayed hypersensitivity that develops after immunization with a protein antigen in Freund's adjuvant.

1. Rich, A., Lewis, M., *Bull. Johns Hopkins Hosp.*, 1932, v50, 115.

2. Moen, J. K., *J. Exp. Med.*, 1936, v64, 355.

3. Aronson, J., *J. Immunol.*, 1933, v25, 1.

4. Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1945, v59, 134.

5. Miller, J. M., Favour, C. B., *J. Exp. Med.*, 1951, v93, 1.

6. Waksman, B. H., *J. Immunol.*, 1953, v70, 331.

7. Rich, A., *The Pathogenesis of Tuberculosis*, Chas. C Thomas, Springfield, 1946, p717.

8. Raffel, S., *Immunity*, Appleton Century Crofts, New York, 1961, p337.

9. Gangarosa, E. J., Inglefield, J. T., Thomas, C. G. A., Morgan, H. R., *J. Exp. Med.*, 1955, v102, 425.

10. Waksman, B. H., Matoltsky, M., *J. Immunol.*, 1958, v81, 220.

11. Mackaness, G. B., *J. Path. and Bact.*, 1952, v64, 429.

12. George, M., Vaughan, J. H., *J. Immunol.*, 1962, v88, 191.

13. Boyden, S. V., The Immunological Response to Antigens of the Tubercle Bacillus in *Progress in Allergy*, S. Karger, Basel, vol. V, 1958.

14. Benacerraf, B., Gell, P. G. H., *Immunol.*, 1959, v2, 53.

15. Salvin, S. B., Smith, R. F., *J. Exp. Med.*, 1960, v111, 465.

16. Cruickshank, C. N. D., *Brit. J. Exp. Path.*, 1954, v35, 609.

17. Tuncman, S., Packalen, T., *Acta Path. et Microbiol. Scand.*, 1959, v46, 361.

18. Metaxas, M. N., *J. Immunol.*, 1955, v75, 333.

19. Marks, J., *J. Path. and Bact.*, 1958, v75, 39.

20. Rebuck, J. W., Yates, J. L., *Am. Rev. Tuberc.*, 1954, v69, 216.

21. Eisen, H. N., Helmreich, E., Kern, M., Metabolic Activities of Isolated Lymph Node Cells in *Mechanisms of Hypersensitivity*, J. H. Shaffer, G. A., Lo Grippo, M. W. Chase, (eds.), Little, Brown & Co., Boston, 1959.

22. Puck, T. T., Marcus, P. I., Cieciura, S. J., *J. Exp. Med.*, 1955, v103, 273.

23. Raffel, S., *Immunity*, Appleton Century Crofts, New York, 1961, p349.

24. Paterson, P. Y., *Discussions in Western Society of Immunologists*, LaJolla, Calif., January, 1961.

Received September 18, 1962. P.S.E.B.M., 1962, v111.

Distribution and Excretion of Aminonucleoside-8-C^{14} in Normal and Nephrotic Rats.* (27842)

CARL S. ALEXANDER, HERBERT T. NAGASAWA AND DOROTHY FILBIN

Medical and Radioisotope Service, Veterans Administration Hospital, and University of Minnesota, Minneapolis

The discovery by Frenk *et al.*(1) and subsequently elaborated by others(2) of the ability of the aminonucleoside (PA) of the antibiotic puromycin to induce the classic nephrotic syndrome in rats as typically seen in the spontaneous disease of children has spurred a large number of investigators to study its mechanism of action in the hopes of understanding the etiology of the natural disease. Rats of different strains were susceptible to the action of this drug whether given subcutaneously or orally(3,4), but mice, guinea pigs, rabbits and dogs were found to be resistant to short term treatments(3). The nephrotoxicity of PA in primates (Rhesus monkeys)(5) and in humans(6) has been recorded.

Bartlett and Shelata using tritiated PA(7) and later with the aid of the more stable carbon-14 labeled material(8) have studied the incorporation of the label into nucleic acids and acid soluble nucleotides of the rat liver and kidney. Wilson *et al.*(9) have compared

* Supported by U.S.P.H.S. grant.