tryptamine well, (as gels did not stain when tryptamine was substituted for benzylamine) and would thus appear to function more like the amine oxidase previously described for plasma. The relationship between these different tissue enzymes and the blood enzyme, as well as the role of the enzymes in the overall celular economy is an intringuing question[17].

[17] This research was supported by NIH research grant No. 1 RO3 MH 20238-01 from the National Institute of Mental Health, and a Faculty Research Grant from Fordham University. We would like to thank R. Blitz and T. Rossi for their excellent technical assistance.

Zusammenfassung. Menschliche Plasmaaminooxydase, bestehend aus verschiedenen Isoenzymen, deren Hauptfraktion in zahl reichen untersuchten parenchymatösen Organen vorhanden ist, wurde mit der Stärkegelektrophorese und Sephadex Säulenchromatographie untersucht. Isoenzyme aus Gewebehomogenaten und Plasma unterscheiden sich.

Nancy Rauch and R. J. Rauch

Department of Biological Sciences, Fordham University, Bronx (New York 10458 USA) and Department of Psychiatry, Albert Einstein College of Medicine, Bronx (New York 10461 USA),
16 August 1972.

Phagocytosis of Latex Particles in vitro: Effects of Antilymphocyte and Antithymocyte Serum

Several investigators have considered the possibility that impairment of phagocytosis contributes to the immunosuppressive capacity of antilymphocyte serum[1-3]. The primary site of action and the mechanism of phagocytic depression is not yet clear, however. Preferential impairment of splenic dendritic macrophages was found in ALS-treated mice by Barth et al.[4], while selective impairment of hepatic phagocytosis was described by Pisano et al.[5]. The finding that ALS-sensitized lymphocytes localized predominantly in the liver[6], raised the possibility that an immunoglobulin component is present in ALS which promotes phagocytosis by the Kupffer cells[7].

The present communication reports the results obtained when monolayers of mouse macrophages were incubated with ALS or ATS in vitro and their phagocytic activity was measured at different time intervals.

Materials and Methods. Horse antimouse thymocyte serum (ATS) was obtained from the Institute of Microbiology and Hygiene of the University of Montreal, through the courtesy of Dr. H. E. Taylor, Medical Research Council of Canada. Rabbit anti-mouse lymphocyte serum (ALS) was purchased from Microbiological Associates, Bethesda, Md. Peritoneal cells were obtained

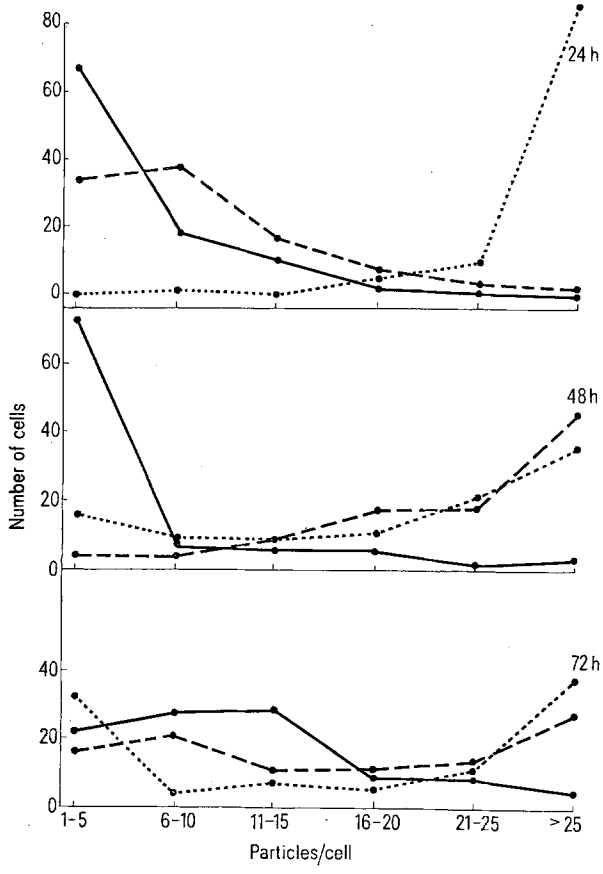

Fig. 1. Particle distribution within peritoneal macrophages after 1, 2 and 3 days in culture. Solid line: 40% ATS in both the culture medium and test medium; broken line: 40% ATS followed by Latex in 40% horse serum; dotted line: control.

Table I. Effect of ALS on the phagocytic activity of macrophages in vitro

Incubation time	Peritoneal macrophages Percent ALS			Spleen macrophages Percent ALS		
	4	20	40	4	20	40
30 min	97	92	70	92	94	69
2 h	79	65	—	76	79	—
48 h	84	99	75	84	88	69
72 h	—	—	95	—	—	98

Results are expressed as percent control values. Control cultures were maintained in medium supplemented with 40% inactivated newborn calf serum.

[1] J. N. Sheagren, R. F. Barth, J. B. Edelin and R. A. Malmgren, J. Immun. *105*, 634 (1970).
[2] N. S. Harris, G. E. Merino and J. S. Najarian, Transpl. Proc. *3*, 797 (1971).
[3] J. C. Pisano, J. T. Patterson and N. R. Diluzio, in *The RES and Immune Phenomena* (Ed. N. R. Diluzio and K. Fleming; Plenum Press, New York 1971), p. 237.
[4] R. F. Barth, R. L. Hunter, J. Southworth and A. S. Rabson, J. Immun. *102*, 932 (1969).
[5] J. C. Pisano, J. T. Patterson and N. R. Diluzio, Proc. Soc. exp. Biol. Med. *132*, 517 (1969).
[6] W. J. Martin and J. F. A. P. Miller, Int. Arch. Allergy *35*, 163 (1969).
[7] M. Rabinovitch, in *Mononuclear Phagocytes* (Ed. R. van Furth; Blackwell Scientific Publications, Oxford 1970), p. 299.

from unstimulated Swiss mice by washing the peritoneal cavity with 2.5 ml medium 199 containing 2 mg/100 ml heparin. 2×10^6 washed cells were resuspended in 1.0 ml medium 199 supplemented with inactivated horse serum (in experiments with ATS) or with inactivated newborn calf serum (in experiments using ALS). Spleen cell suspensions were prepared by gently forcing minced splenic tissue through a nylon sieve. Cover slip cultures were established with 5×10^6 washed cells per Leighton tube. Non-adherent peritoneal and splenic cells were removed 2 and 24 h after the initiation of the cultures, respectively, and fresh medium containing ATS or ALS was added to the rinsed cultures. After the required incubation period, the used medium was removed, the cultures rinsed and 1.0 ml fresh medium containing 1.44×10^9 washed Latex particles (1.09 µm; Dow Chemical Co., Midland, Michigan) was added. Leighton tube coverslip cultures of strain L-cells, initiated with 1.0×10^6 cells, were used in comparative experiments. Percentage phagocytosis was calculated scoring a minimum of 300 cells in each Giemsa-stained slide for the presence of intracellular Latex particles. Particle distribution was determined by counting the number of particles in 100 cells on each slide.

Results. The effects of ALS on the phagocytic activity of macrophages in vitro are summarized in Table I. Transient depression of particle phagocytosis was observed. At 4% and 20% ALS concentration, decrease in phagocytic activity became evident after 2 h incubation, while only 30 min contact was necessary for the maximum effect to develop in the presence of 40% ALS. Both peritoneal and splenic mononuclear phagocytes recovered phagocytic activity to the level of the controls by 48 h in the continued presence of 4% and 20% ALS; at higher ALS concentration recovery was complete by 72 h. Removal of ALS from the culture medium after 24 h incubation did not influence the rate of recovery.

Evaluation of the phagocytic capacity of cultured macrophages by determination of particle distribution within the phagocytic population confirmed the transient nature of ATS-induced phagocytic depression (Figure 1). After 72 h incubation, no significant difference was found between ATS-treated and control cells.

ALS also influenced the phagocytic capacity of strain L-cells confirming the observations that the activity of ALS is not directed against antigenic determinants specific to lymphocytes alone[8,9]. After 24 h incubation in 10% and 20% ALS, most cells contained less than 10 particles (Table II). Continued incubation in the presence of ALS lead to recovery of phagocytic capacity, reaching the levels of the respective control cultures after 3 days.

In a series of experiments, mice received 0.25 ml of ATS i.p., either twice (Group A) or 4 times (Group B) on consecutive days. On the day following the last ATS injection, 1.44×10^9 Latex particles suspended in medium 199 were given i.p. (Group A) or i.v. (Group B). A 3rd group of mice received only ATS treatment (Group C) while control animals were injected with equivalent volumes of inactivated horse serum. The mice were sacrificed 30 min after the Latex injection and peritoneal and splenic macrophage cultures were established. The cultures obtained from Group C mice were exposed to Latex particles on the 2nd and 4th day of culture. The results illustrated in Figure 2 show that in vivo administered ATS did not depress the phagocytic activity of either peritoneal or splenic cells. On the contrary, splenic macrophages appeared to have been stimulated by ATS treatment. These results do not exclude the possibility that within 48 h following the initial administration of ATS transient depression of phagocytic activity occurred.

Discussion. The results of the present study indicate that ATS or ALS-induced impairment of phagocytic activity is of short duration. It appears unlikely that depression of phagocytosis contributes to the sustained action of ALS[10–12]. The transient nature of ALS- induced phagocytic depression in vitro and the stimulation of splenic macrophage phagocytosis following in vivo ATS administration suggest that antibodies present in ATS may react with macrophage membrane receptors[13] with subsequent enhancement of endocytosis and increased

Table II. Particle distribution in strain L-cells following 24 h exposure to ALS

Incubation medium	Percentage cells containing Latex particles per cell 1–5	6–10	11–15	16–20	21–25	>25
2% ALS	3	6	15	16	26	34
Control	6	6	9	12	13	54
10% ALS	82	13	3	2	0	0
Control	6	10	6	12	6	60
20% ALS	77	18	3	2	0	0
Control	3	16	16	11	16	38

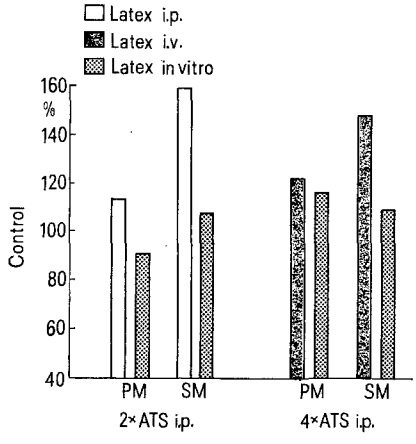

Fig. 2. Phagocytosis of Latex particles by peritoneal (PM) and splenic macrophages (SM) following i.p. administration of ATS.

[8] R. H. LEVEY and P. B. MEDAWAR, Proc. natn. Acad. Sci. *56*, 1130 (1966).
[9] S. V. JOOSTE, in *Proceedings of Symposium on Organ Transplantation in South Africa* (Ed. A. J. BRINK; University of Stellenbosch Medical School, Bellville 1967).
[10] R. H. LEVEY and P. B. MEDAWAR, in *Antilymphocytic Serum* (Ed. G. E. W. WOLSTENHOLME and M. O'CONNOR; Ciba Foundation Study Group No. 29, J. & A. CHURCHILL Ltd., London 1967), p. 72.
[11] M. J. B. CHARE and J. L. BOAK, Clin. exp. Immun. *6*, 655 (1970).
[12] R. GALLILY and M. GORNOSTANSKY, Immunology *22*, 431 (1972).
[13] H. HUBER, G. MICHLMAYR and H. H. FUDENBERG, Clin. exp. Immun. *5*, 607 (1969).

turnover of cell membrane constituents[14]. Participation of macrophage membrane receptors was also suggested in ALS-induced depression of macrophage migration in vitro[15] since the effect was substantially reduced by trypsinization[16].

[14] Z. A. Cohn and E. Parks, J. exp. Med. *125*, 1091 (1967).
[15] J. Svejcar, J. Pekarek and J. Johanovsky, Z. Immun. Forsch. exp. Ther. *142*, 166 (1971).
[16] Acknowledgments. The author is grateful to Mrs. Annemarie Klinkenberg for able technical assistance. This work was supported by the Medical Research Council of Canada.

Résumé. Etude des effets des sérums antilymphocytes sur la phagocytose in vitro par les macrophages de la rate et du péritoine de la souris. L'inhibition partielle de la phagocytose a été transitoire; les cellules macrophages ont récupéré leur capacité fonctionelle deux jours après l'administration de sérum antilymphocyte.

Susan Tolnai

Department of Histology and Embryology, Faculty of Medicine, University of Ottawa, Ottawa, K1N 6N5 (Ontario, Canada), 17 July 1972.

Inhibition of Epinephrine-induced Glycogen Phosphorylase Activation by *Bordetella pertussis* Vaccine in Rats

It has been previously shown that hyperglycemic response to epinephrine was inhibited by *Bordetella pertussis* vaccine (BPV)[1,2], and this mechanism may be involved in the development of increased sensitivity to anaphylactic shock and shock mediators following BPV treatment. The purpose of the present study was to investigate how BPV influences epinephrine-induced liver and muscle glycogen phosphorylase activation. Since in BPV-treated rats the normal hyperglycemic response was restored by prednisolone treatment[2], the effect of prednisolone on the disturbed glycogen phosphorylase activation was also investigated.

Materials and methods. Wistar male rats (150–200 g) were maintained on a standard diet and drinking water was given ad libitum. Experiments were performed on fed animals at 09.00 h to insure adequate glycogen levels. BPV was administered i.p. in a single dose of 3×10^{10} organisms. Some groups of normal and BPV-sensitized rats were treated with 25 mg/kg prednisolone succinate (Organon) s.c. twice daily over a period of 3 days. The last dose of prednisolone was injected 12 h before phosphorylase examination.

All rats were anaesthetized with pentobarbital 30 min before sacrificing. Liver and muscle samples were taken 10 min after the i.p. injection of epinephrine (0.1 mg/kg) or phys. saline. Active liver phosphorylase was assayed according to the procedure of Hedrick and Fisher[3]. Gastrocnemius muscle samples were prepared as described by Schaeffer et al[4]. Total and active muscle phosphorylase levels were determined by assaying with and without 1 m*M* AMP, respectively, according to the method of Hedrick and Fisher[3]; and besides active phosphorylase levels, the ratios of active to total enzyme activities are indicated in Table II. Phosphorylase activities are expressed as mmol released inorganic phosphate/g tissue/h (mmol $P_i/g^{2'}h^{-1}$). The results are statistically evaluated by Student's *t*-test.

Results and discussion. BPV, prednisolone, or BPV + prednisolone did not influence active liver phosphorylase levels (Table I). Epinephrine caused about a 100% increase of active phosphorylase level in normal rats. The response to epinephrine was significantly inhibited by BPV pretreatment. Prednisolone alone caused no changes, however, in BPV inoculated rats the sensitivity to epinephrine was restored by prednisolone.

BPV alone did not alter the level of active and total muscle phosphorylase (Table II). Prednisolone treatment resulted in an increase both in normal and in BPV-treated rats; however the ratio of active to total phosphorylase was not significantly influenced. Following epinephrine administration an activation of muscle phosphorylase could be observed in normal rats as indicated by the increase of the ratio of active to total enzyme activity. This effect of epinephrine was merkedly inhibited by BPV pretreatment. The sensitivity of BPV-treated rats to epinephrine was partially restored by prednisolone.

The diminution of epinephrine-induced hyperglycemia in BPV-treated rats[1,2] may be due to the disturbance of insulin secretion and to the changes of liver glycogen metabolism. An increased sensitivity of insulin secretion mechanism in BPV-treated rats was demonstrated by our previous results[5]. Recently Kreutner et al.[6] reported that BPV did not influence the effect of epinephrine on liver glycogen synthetase, whereas it inhibited epinephrine-induced activation of liver glycogen phosphorylase. The latter findings are supported by our present observations. In addition, a diminished response of muscle phosphorylase to epinephrine was demonstrated in this paper.

The inhibited response of BPV-treated rats to epinephrine is probably due to an impairment occurring at any one

Table I. Effect of epinephrine on active liver phosphorylase level in normal, BPV, prednisolone and BPV + prednisolone-treated rats

	Phosphorylase activity mmol $P_i/g^{2'}h^{-1} \pm$ SE	
	Phys. saline	Epinephrine
Normal	1.18 ± 0.06 (8)	2.37 ± 0.12^{a} (9)
BPV	1.28 ± 0.07 (10)	$1.76 \pm 0.06^{b,c}$ (9)
Prednisolone	1.25 ± 0.10 (8)	2.40 ± 0.12^{a} (9)
BPV + prednisolone	1.27 ± 0.11 (8)	2.26 ± 0.10^{a} (9)

[a] $p < 0.001$; [b] $p < 0.01$ related to values obtained after phys. saline. [c] $p < 0.01$ if the increase caused by epinephrine in normal rats was related to that detected in BPV-treated animals. Numbers in parentheses represent the number of animals in each group.

[1] A. Gulbenkian, L. Schobert, C. Nixon and I. I. A. Tabachnick, Endocrinology *83*, 885 (1968).
[2] L. Muszbek, B. Csaba, K. Kovács and Z. Csizér, Acta allerg. *26*, 463 (1971).
[3] J. L. Hedrick and E. H. Fisher, Biochemistry *4*, 1337 (1965).
[4] L. D. Schaeffer, M. Chenoweth and A. Dunn, Biochim. biophys. Acta *192*, 304 (1969).
[5] L. Muszbek, B. Csaba and J. Csongor, Acta allerg., in press.
[6] W. Kreutner, A. Gulbenkian and I. I. A. Tabachnick, Biochem. Pharmac. *19*, 2561 (1970).