number of possible explanations which have not yet been experimentally considered are the following: (1) variability in the amount of 'cold' or endogenous TdR pool to dilute the labeled thymidine, (2) variability in the proportion of spleen cells capable of undergoing mitosis (and therefore H^3-TdR → DNA) without PHA stimulation, and (3) variability of cells to respond to fetal calf serum as an antigen.

The mechanisms by which trypsin enhances H^3-TdR incorporation into spleen cell cultures are not known. A recent observation[9] on human peripheral blood lymphocytes indicating that trypsinization acts to inhibit thymidine incorporation would appear to be inconsistent with our present findings. However, our studies indicated that higher than optimal concentrations of trypsin lowered the ability of spleen cultures to respond to PHA. Studies have shown that PHA or PWM[10,11] stimulated lymphocytes possessed fine structural alterations as compared with non-PHA treated lymphocytes; in particular, these studies have indicated that there was an increase in vacuoles containing acid phosphatase, an enzyme which is closely associated with lysosomes. These studies, together with our studies on trypsin action on isolated spleen cells, are consistent with the notion that a certain degree of injury, perhaps, to releasing lysosomal enzymes, may be important in the induction of blastogenesis. HIRSCHHORN and HIRSCHHORN[12] suggested that rupture of a few lysosomes of a small lymphocyte may be an early stimulus to derepression of this cell type[13].

Résumé. On a étudié l'action de la phytohémagglutinine (PHA) sur la stimulation de l'incorporation de thymidine tritiée à la DNA de cellules spléniques de rats, en cultures à court terme. Il a été possible de stimuler la synthèse de la DNA dans ce type de cultures au moyen de la trypsinisation des cellules dispersées avant l'addition de la PHA. Les variations relativement faibles observées dans ce type d'essai vont faciliter les études des mécanismes cellulaires et biochimiques associés à la stimulation par la PHA.

E. M. UYEKI and V. LLACER

Department of Pharmacology, University of Kansas Medical Center, Kansas City (Kansas 66103, USA), 15 September 1967.

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The Turbidimetric Evaluation of Platelet Aggregation Caused by Bacterial Lipopolysaccharide

Aggregation of blood platelets occurs in the presence of adenosine diphosphate (ADP), thrombin, collagen, fatty acids, antigen-antibody complexes, and bacterial lipopolysaccharide (endotoxin). ADP causes aggregation initiated by several of these agents[1,2]. The dynamics of aggregation and the effects of inhibitory substances have been determined for many aggregating materials using a turbidimetric technique[3]. That technique, however, does not appear to have been used to characterize aggregation by bacterial lipopolysaccharide (LPS). The results of such a study are described in this report.

Sprague Dawley rats weighing 350 g were anesthetized with diethyl ether by inhalation. Aortic blood obtained through polyethylene cannulae was collected in plastic (Nalgene[4]) tubes into $^1/_{10}$ volume of heparin sodium[5] in imidazole buffered (pH 7.4) sodium chloride solution (0.154 *M*). Unless otherwise indicated, heparin was diluted ($^1/_{100}$) in buffer, the final heparin concentration being 1 U/ml of whole blood. Blood was centrifuged for 7 min at 175 *g* (4 °C) to obtain platelet rich plasma (PRP, approximately 600,000 platelets/mm³). PRP was stored at 10–15 °C until used. When necessary, PRP was diluted with platelet poor plasma. The study of aggregation was completed 30 min after collection of blood. All experiments were paired; 2 samples of PRP were used from each rat, one being a suitable control, the other an experimental sample. In studies using varying amounts of heparin, blood was collected in separate tubes. Platelet aggregation was studied turbidimetrically at 37 °C with a Bausch and Lomb spectrophotometer as described[6] although optical density changes were determined at 620 nm. *Escherichia coli* LPS (control 0127:B8) was used in all studies. LPS was dissolved in imidazole buffered saline (concentration of 0.5 mg/ml), and 0.1 ml was added to 2.0 ml of PRP. Before addition of LPS, all samples were warmed at 37 °C for 2 min, and the optical density was determined at 1 min intervals for 3 min. Following addition of LPS or IBS, samples were stirred continuously for 5 min, with O.D. being recorded at 15 sec intervals. Thereafter, O.D. was recorded every min for 5 min, and the experiment terminated.

Addition of LPS to PRP caused an initial slow decrease followed by an accelerated phase of decrease in O.D. The onset of the accelerated decrease was as early as 75 sec and as as late as 270 sec after LPS. The slower phase of decrease generally occurred during the first 10% of the fall in O.D. Mean total changes in O.D. in control and experimental samples during the 10 min period were 5% and 61% respectively (Figure 1). In 10 rats the final heparin concentration was 10 u/ml, and 1 u/ml in another 10 rats. The mean initial O.D. of PRP in the former group was 0.70, and 0.68 in the latter group. Using 10 u/ml the accelerated decrease of O.D. was retarded (Figure 1), but until 135 sec after LPS mean differences in O.D. in the 2 groups were $\leqslant 6\%$. Statistically significant differences in mean values were observed 4 min after addition of LPS

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($p < 0.05$); at 5 min and at later intervals the differences were highly significant ($p < 0.001$).

The effect of adenosine on ADP-induced aggregation was evaluated. Using $5 \times 10^{-4} M$ adenosine and $7 \times 10^{-8} M$ ADP, a diminution of aggregation occurred (Figure 2) within the first 60 sec after ADP ($p < 0.01$). Ninety sec after ADP the O.D. in adenosine pretreated samples and samples with ADP alone were more nearly equal. Two of 10 samples in which ADP alone was added had an O.D. of 0.4–0.6 throughout the period of observation, while the O.D. of the other 8 samples returned to 95–100% of the control, which was observed in samples pretreated with adenosine. Adenosine ($5 \times 10^{-4} M$) also was added to PRP (5 experiments), incubated for 10 min, and LPS added. Paired control samples were treated similarly with saline instead of adenosine. The changes in O.D. are recorded in Figure 3. The final decrease in O.D. was unchanged in the group which received adenosine, but differences between adenosine and saline pretreated groups were observed during the phase of accelerated aggregation (2–5 min). The differences in O.D. were statistically significant 4 min, and 4 min 15 sec after addition of LPS ($p < 0.05$).

5-Hydroxytryptamine (5-HT, final concentration 1.5 µg/ml) was added to rat PRP. A variable decrease in O.D. was observed. Of 4 experiments 2 showed a decrease in O.D., beginning 3–4 min after 5-HT; no changes were observed in 2. 1.0 µg/ml and 0.5 µg/ml produced no changes in O.D. (4 experiments). Addition of 5-HT (1.0 µg/ml) to PRP 30 sec prior to addition of LPS appeared to accelerate the decrease in O.D. slightly. Thus, 3, 4, 5, and 6 min after LPS the differences in means in the 2 groups were 10, 12, 9 and 8%. Those differences were not significant. In addition, the use of 1.5 µg/ml of 5-HT did not increase the magnitude of aggregation by LPS.

These experiments suggest that thrombin and ADP contribute to late in vitro aggregation caused by LPS. The effects of the 2 compounds were not observed initially while aggregation was occurring; and within 4 min after LPS, the extent of aggregation was not statistically influenced by increased amounts of heparin, in accord with earlier observations in vivo[7]. The occurrence of aggregation soon after LPS was similar to that observed after addition of immune complexes[8]. Of interest was the observation that adenosine decreased the initial fall in O.D. after addition of ADP in an amount to obtain a minimal response. This observation was made in the presence of small amounts of heparin (1 u/ml). With larger amounts (15 u/ml), adenosine has been found not to decrease ADP induced aggregation[9] though it does so with citrated[9] or native[10] rat PRP. The lack of responsiveness of selected test systems in the rat has been noted before[11]. Thromboplastin generation has been reported

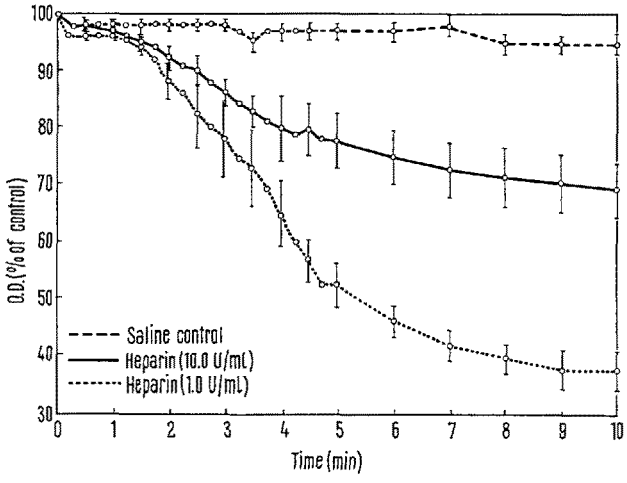

Fig. 1. Changes in optical density of rat platelet rich plasma. With saline (control) samples 0.1 ml of 0.15 M sodium chloride was added to 2.0 ml of heparinized platelet rich plasma. LPS (final concentration 25 µg/ml) was added to the experimental samples, collected with a final concentration of heparin of 1.0 or 10.0 USP U/ml whole blood. The results are expressed as the mean ± S.E. mean.

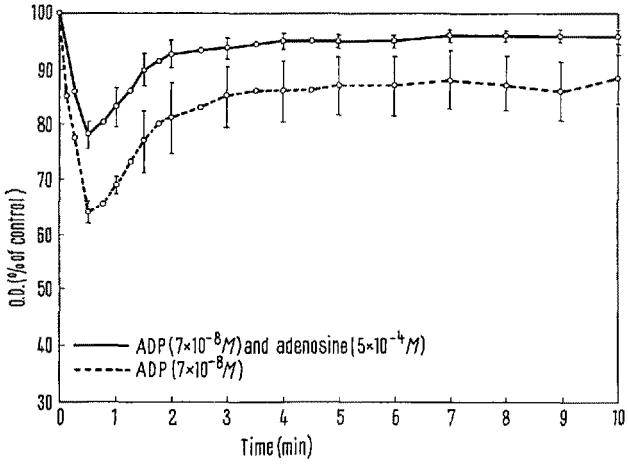

Fig. 2. Changes in optical density of rat platelet rich plasma caused by ADP (broken line), compared to optical density changes caused by ADP following incubation with adenosine (solid line).

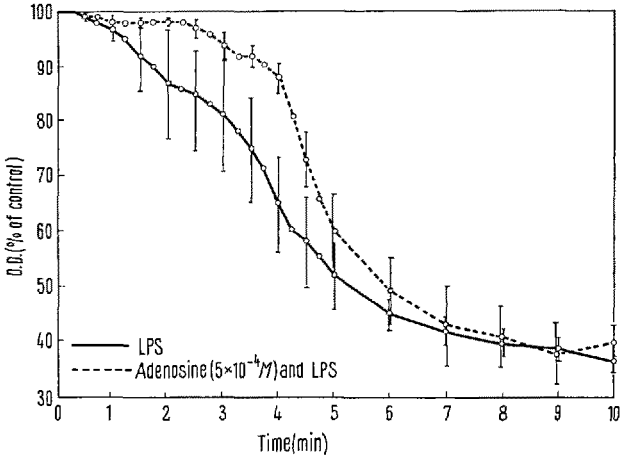

Fig. 3. Changes in optical density of rat platelet rich plasma after addition of LPS alone (solid line) or LPS following incubation with adenosine. The final concentration of LPS was 25 µg/ml in both instances.

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to be rapid in rat plasma[12] and LPS is believed to activate factor XI (PTA)[13]. Heparin is known to block activated factor IX, and part of the effect of heparin in slowing aggregation in vitro may be related to its action in the intrinsic coagulation system. Although 5-HT is known to cause aggregation of human platelets, its action on rat platelets was variable; and it did not appear to increase aggregation of platelets by LPS[14].

Zusammenfassung. Die Aggregation von Blutplättchen durch *Escherichia coli* Endotoxin wurde untersucht: Die Blutplättchenanhäufung erweist sich anfänglich als langsam, dann aber als sehr schnelle Abnahme der optischen Dichte eines blutplättchenreichen Plasmas. Die schnelle Abnahme der optischen Dichte konnte durch Heparin, und zwar nur in grösserer Menge, unterdrückt werden. Der Beginn der schnellen Ansammlung wurde durch Adenosin verzögert, wobei aber Adenosin keine Wirkung auf den Grad der Anhäufung durch das Endotoxin hatte. Die Ergebnisse weisen darauf hin, dass in vitro Thrombin und Adenosindiphosphat die Thrombozytenaggregation durch Endotoxin unterstützen.

R. B. Davis[15]

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The Occurrence of Dopamine and Noradrenaline in the Tubero-Hypophysial System

It has been found with a specific fluorescence method that a great number of primary catecholamines containing nerve fibres terminate around the hypophysial portal capillaries in the median eminence[1-3]; the monoamine involved is mainly dopamine, but some noradrenaline is also present[1]. The site of origin of the nerve fibres forming the tubero-hypophysial system seems to be the arcuate and periventicular nuclei[2,4]. In the present work, the content of dopamine and noradrenaline in the median eminence and infundibular stem region, the mediobasal hypothalamus and the rest of hypothalamus of man, pig and cattle was determined chemically. The post-mortem changes in the content of the amines were studied as well.

Material and methods. The material consisted of 9 men, 40 pigs and 30 cows. The brains were dissected from pig within 5 min, from cattle within 15 min and from man 6–18 h after death. The human brains were selected in the autopsy room from patients not having suffered from cerebral or mental disease, but having died either from infection or from diseases of the circulatory system. Tissue samples of the median eminence-infundibular stem region, mediobasal hypothalamus and the rest of the hypothalamus were pooled from 5 pigs and 3 cows and assayed as 1 sample, whereas the tissue samples from man were assayed individually. The tissues were homogenized immediately in perchloric acid, centrifugated and stored frozen until analyzed, usually within a few days. Dopamine was determined by the method of Carlsson and Waldeck[5] and noradrenaline as described by Crout[6].

In order to study the post-mortem changes 30 rats were killed at 08.00 and the brains were dissected immediately, 1, 12 and 24 h after death. Before autopsy the killed rats were stored at + 4 °C. The content of dopamine and noradrenaline in the brain excluding the cerebellum was determined as described above.

Results and discussion. The content of dopamine and noradrenaline in the median eminence and infundibular stem region, in the mediobasal hypothalamus and in the rest of hypothalamus of man, pig and cattle are summarized in Table I. The highest values of dopamine were found in the median eminence and infundibular stem region and that of noradrenaline in the mediobasal hypothalamus although there was some species difference.

As shown in Table II, the content of noradrenaline in the rat brain decreased significantly already 1 h after death, whereas the significant decrease of dopamine content occurred after 24 h.

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Table I. Content (μg/g) of dopamine (DA) and noradrenaline (NA) in the median eminence and infundibular stem region (ME), the mediobasal hypothalamus (MBH) and the rest of hypothalamus (RH) of man, pig and cattle.

Species	ME		MBH		RH	
	DA	NA	DA	NA	DA	NA
Man						
Mean S.E.M. No.	0.24 ± 0.101 (9)	1.66 ± 0.349 (9)	0.18 ± 0.075 (9)	1.34 ± 0.163 (9)	0.34 ± 0.046 (9)	0.45 ± 0.092 (9)
Pig						
Mean S.E.M. No.	0.85 ± 0.135 (8)	0.78 ± 0.086 (8)	0.14 ± 0.027 (8)	1.97 ± 0.146 (8)	0.13 ± 0.024 (8)	0.61 ± 0.054 (8)
Cattle						
Mean S.E.M. No.	0.43 ± 0.095 (10)	0.91 ± 0.124 (10)	0.27 ± 0.066 (10)	1.71 ± 0.141 (10)	0.07 ± 0.007 (10)	0.76 ± 0.086 (10)