Test compound[a]	Effect on platelet aggregation induced by	
	$9.1 \times 10^{-7} M$ ADP in the rat	$1.8 \times 10^{-7} M$ ADP in the guinea-pig
Ethacrynic Acid	O[b]	O
Meralluride	I[c]	I
Mersalyl	I	I
p-Chloromercuribenzoic acid	I	I
Methylmercuric chloride	S[d]	S

[a] Platelet rich plasma was preincubated for 20 min in the presence of $7 \times 10^{-3} M$ ethacrynic acid, $2 \times 10^{-3} M$ meralluride, $1 \times 10^{-3} M$ mersalyl, $2.5 \times 10^{-4} M$ *p*-chloromercuribenzoic acid and $1 \times 10^{-4} M$ methylmercuric chloride. Spectrophotometer determinations were carried out at a platelet dilution corresponding to 300,000 platelets/mm³ for rat and 150,000 platelets/mm³ for guinea-pig. [b] O, no effect. [c] I, complete inhibition. [d] S, caused aggregation in the absence of ADP.

partment cover. A description of this device is in press[11]; briefly it consists of 4 replaceable glass rods, 2 per cuvette, positioned so that the light beam can pass between them, a solenoid which oscilates the glass rods at 60 c/sec and a powerstat which controls the amplitude of the oscillations.

Compounds were obtained from the following sources: Adenosine diphosphate and *p*-chloromercuribenzoic acid (Mann), mersalyl (Sigma), ethacrynic acid (Merck Sharp and Dohme), meralluride (Lakeside Lab.) and methylmercuric chloride (Alpha Inorganics).

Results and discussion. The Table shows the effect of the test compounds on ADP-induced platelet aggregation in both rat and guinea-pig. PCMB and methylmercuric chloride were included as reference compounds because their reactions with sulfhydryl groups and effects on platelet aggregation have been previously investigated in other species[4]. The presence of meralluride and mersalyl resulted in complete inhibition of ADP-induced aggregation, while ethacrynic acid, the other diuretic possessing sulhydryl binding activity, had no effect. The stimulatory effect observed with $1 \times 10^{-4} M$ and $1 \times 10^{-3} M$ (not illustrated) methylmercuric chloride is of interest in view of the report of ROBINSON et al.[12] that complete inhibition of canine platelet aggregation was observed with methylmercuric nitrate concentrations of $6.3 \times 10^{-5} M$ or greater. It thus appears that inhibition of platelet aggregation cannot be predicted solely on the basis of organic molecules containing mercury or possessing the capacity to bind sulfhydryl groups.

Commercial preparations of the organic mercurial diuretics, mersalyl and meralluride, contain large amounts of theophylline added to prevent breakdown of the organomercuric complex. This compound has been shown to inhibit the c-AMP phosphodiesterase enzyme[13], thus effectively maintaining high levels of c-AMP in the platelets. Since high platelet levels of c-AMP are associated with inhibition of aggregation[14], the possibility must be considered that the presence of theophylline in these preparations would potentiate the platelet aggregate inhibiting effect of these mercurial diuretics. Experiments are now in progress to determine whether these diuretics also affect the synthesizing enzyme (adenyl cyclase) or the degrading enzyme (c-AMP phosphodiesterase) involved in the regulation of c-AMP levels in platelets.

Résumé. L'agrégation des plaquettes, induite par l'ADP exogène, fut observée par turbidimétrie en mesurant les variations de la densité optique. Le méralluride, le méersalyl et le PCMB préincubés avec les plaquettes de rat et de cobaye préviennent l'agrégation, tandis que l'acide éthacrynique n'a aucun effet. Par contre, le chlorire méthylique de mercure la provoque en l'absence d'ADP.

R. E. A. GADD, S. CLAYMAN and D. HÉBERT

Research Laboratories, Food and Drug Directorate, Tunney's Pasture, Ottawa 3 (Ontario, Canada), 10 May 1971.

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Inhibition of Lymphocyte Transformation by Guanidinosuccinic Acid, a Surplus Metabolite in Uremia

When patients with renal failure become uremic, cellular immunity is suppressed and homograft rejection is delayed[1,2]. This alteration in immunity probably is related to an abnormality of lymphocyte function. Although washed lymphocytes from uremic guinea-pigs transform normally under stimulation from phytohemagglutinin (PHA)[3], several investigators have observed that blood plasma from uremic patients inhibits the transformation of lymphocytes to lymphoblasts in vitro[4,5]. Recently a dialyzable uremic toxin has been suggested to explain inhibition of PHA-stimulated lymphocyte transformation[6]. Because the lymphocyte plays an important role in cellular immunity, we are presently attempting to identify dialyzable uremic toxins which might alter immunity by inhibiting lymphocyte transformation.

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Effect of guanidinosuccinic acid on incorporation of tritiated thymidine by normal human peripheral blood lymphocytes (cpm per 10^3 lymphocytes)

Experiment	No additions	Phytohemagglutinin	Phytohemagglutinin and guanidinosuccinic acid
1	65	7,900	1,062
2	85	6,413	1,663
3	45	5,969	1,793
4	168	10,260	1,149
5	43	25,356	13,241
6	12	10,753	6,709
7	83	36,393	29,317
8	46	20,602	16,100
9	64	29,823	13,213
10	150	39,407	20,559
11	132	3,081	1,446
12	6	11,129	9,063
13	127	36,497	23,003
14	90	3,459	1,606
15	22	8,673	5,391

The list of suspected uremic toxins includes 100 or more compounds[7]. From this list, we chose guanidinosuccinic acid (GSA) to study first. GSA is an unusual product of nitrogen metabolism that accumulates in the blood of uremic patients[8]; it is dialyzable, and it is already implicated as a cause of the platelet abnormality associated with uremia[9]. We found that GSA inhibits PHA-stimulated lymphocyte transformation.

For our studies, venous blood was collected in heparinized syringes. The blood was allowed to sit for 3 h to permit erythrocytes to settle out, after which the cell-rich plasma was removed and diluted with tissue culture medium[10] to yield a suspension of 0.75×10^6 lymphocytes per ml. Aliquots of these suspensions were incubated either without any additions, with PHA[11], or with GSA[12] in a final concentration of $2.6 \times 10^{-6} M$ and PHA for 105 h at 37 °C prior to the addition of 1 µci of tritiated thymidine[13] per ml of incubation mixture. The incubations were continued for another 18 h and then the cells were harvested by centrifugation at $1400 \times g$ for 10 min. The amount of tritiated thymidine in the DNA of the cell pellet was measured by a previously described method[3]. The Table summarizes the results.

Our results confirmed the variation that may occur in the incorporation of tritiated thymidine by lymphocytes from different subjects under similar conditions. Nevertheless, it is apparent that GSA inhibited incorporation of tritiated thymidine in each of the 15 lymphocyte preparations. On the average, 44% inhibition was observed. Higher concentrations of GSA ($2.6 \times 10^{-5} M$ and $2.6 \times 10^{-4} M$) were tried, but they did not increase the extent of inhibition. Perhaps a subpopulation of lymphocytes is resitant to the effect of GSA.

Microscopic examination of lymphocytes incubated in the presence of GSA confirmed that the cells were viable as determined by trypan blue by exclusion and that the extent of transformation was compatible with the extent of incorporation of tritiated thymidine. This is in agreement with observations of others demonstrating that incorporation of tritiated thymidine is an adequate measure of transformation.

GSA is undetectable in serum from normal subjects but is present in high concentration in serum from uremic patients. For a group of 18 uremic patients, COHEN[8] reports a mean value for serum GSA concentration of $1.6 \times 10^{-4} M$, which is in great excess of the concentration that will inhibit PHA-stimulated transformation of susceptible lymphocytes in vitro. Therefore, we suggest that GSA is one of the compounds responsible for the alteration in cellular immunity associated with uremia.

Zusammenfassung. Guanidinbernsteinsäure hemmt die durch Phytohämagglutinin stimulierte Aufnahme von ^{3}H-Thymidin zu 44%.

R. G. SLAVIN and C. D. FITCH

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[10] The tissue culture was prepared by diluting 10 ml of TC 199 10 X to 100 ml with sterile, distilled water and adding 500 units of heparin, 10,000 units of penicillin G, and 10 mg of streptomycin. The pH of the medium was adjusted to 7.3 with $NaHCO_3$.
[11] To each four milliliters of incubation suspension were added 0.1 milliliter of phytohemagglutininin, PHA-M from Difco Laboratories, Detroit, Michigan.
[12] Purchased from Mann Research Laboratories, New York, New York.
[13] Specific activity 2.78 mCi per mg, New England Nuclear Corporation.

Aminosäurenanalyse des Ferritins bei primärer und sekundärer Lungenhämosiderose

Die primäre Lungenhämosiderose (LH), die als idiopathische LH[1-4] und bei gleichzeitiger Glomerulonephritis als Goodpasture-Syndrom (GPS)[1,4,5] bekannt ist, nimmt im Eisenstoffwechsel eine Sonderstellung ein. So ist insbesondere die Irreversibilität[2-4,6] des teilweise in die elastischen Fasern des Lungengerüstes inkrustierten Eisens[7] auffällig. Der Gesamtorganeisengehalt liegt gewöhnlich höher als der bei primärer Siderophilie (Hämochromatose) und der Serumeisengehalt ist erniedrigt. Es wird vermutet, dass eine besondere, möglicherweise mit den sauren Mukopolysacchariden in Zusammenhang stehende Form der Eisenfixation[7] vorliegt, weswegen Untersuchungen des Depoteisens Ferritin sinnvoll erscheinen.

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