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BLOOD CONDITIONS AND LYSOZYME ACTION IN THE APOSYMBIOTIC COCKROACH

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Abstract—Blood volume, blood coagulation time, and volume of free blood flow increased following the injection of lysozyme into F_1 aposymbiotic adult male cockroaches derived from parents fed a mixture of 1% aureomycin, terramycin, and sulphisoxazol. These differences were similar to those in lysozyme-treated symbiotic insects.

The total blood cell count in the aposymbiotic insect was lower than in the symbiotic and, following treatment with lysozyme, was not similarly reduced; in relation to blood volume it may have been actually increased.

The blood of the aposymbiotic cockroach had a high Na^+/K^+ ratio which, contrary to the increase that occurred in the symbiotic insect, was not significantly changed by lysozyme injection.

Lysozyme altered the amino acid picture of the aposymbiotic insect but to a lesser degree and involved fewer amino acids than in the symbiotes. However, the *untreated* aposymbiotes had three times as high a concentration of total amino acids as the normal adult male. Eleven of the sixteen amino acids quantitated had increases of not less than 100 per cent, with isoleucine increased by almost 700 per cent, leucine 600 per cent, alanine 550 per cent, valine 450 per cent, glycine 250 per cent, proline 200 per cent, threonine and serine 150 per cent, lysine and histidine 150 per cent, and arginine 100 per cent. Aspartic and glutamic acids showed slight or no increase, while methionine and phenylalanine were reduced by 20 per cent and tyrosine by 60 per cent. The aposymbiotes were further distinguished by a high concentration of homoserine, which amino acid has not been found in the symbiotic cockroach. The sustained methionine level suggests that changes in the metabolism of the sulphur amino acids are probably not as significant as they are in *Blattella germanica*, and does not explain the high concentration of homoserine; homoserine may rather be associated with the high level of isoleucine.

The high level of amino acids in the aposymbiotic cockroach is thought to fill more than an osmoregulatory function, and may contribute to the defence of the organism against infection. The concentrations and ratios of the amino acids of the aposymbiotic American cockroach differed radically from the xenic type, and also from the aposymbiotic *B. germanica*. These distinctive characteristics suggest that the bacterial symbionts perform different functions in different species of cockroach, and may themselves constitute separate species.

The changes induced by lysozyme in the cockroach are attributed to the active enzyme, and their occurrence in the aposymbiote tends to strengthen the validity of the suggestion put forward earlier that lysozyme may play a significant role in inflammation.

INTRODUCTION

MYCETOCYTES of the fat body of the cockroach contain bacterial symbionts (BLOCHMANN, 1887) which are transmitted through the larval oöcytes (GIER, 1936). The mycetocytes occur in large numbers (WHARTON, 1968), and the bacterial symbionts, as they migrate, are thought to exchange metabolic products with the microvilli of the oöcytes (BUSH and CHAPMAN, 1961; MALKE and BARTSCH, 1966). Because of functional defects that result from their elimination they have been considered important factors in the growth and reproduction of the insect (BROOKS and RICHARDS, 1955).

The amino acids have been studied in symbiont-bearing cockroaches by PRATT (1950), STEVENS (1961), TODD (1958), HENRY and BLOCK (1960, 1961), Block and Henry (in HENRY, 1962), MALKE and SCHWARTZ (1966), and ourselves (WHARTON and LOLA, 1969b), and in aposymbiotic cockroaches by HENRY (1962) and MALKE and SCHWARTZ (1966). The first authors used aposymbiotic *F*₁ larvae of *Blatella germanica* derived from adults that had been fed 0.1% aureomycin continuously throughout their developmental period, while Malke and Schwartz used American larvae that had been injected with lysozyme some months previously. Aposymbiotic *B. germanica* synthesized much less of the amino acids generally than the normal insect, and very little tyrosine and phenylalanine and those amino acids—arginine, leucine, lysine, isoleucine, threonine, and valine—which are considered essential for this cockroach (GORDON, 1959). *Periplaneta americana* larvae in which most of the symbionts had been destroyed by lysozyme (MALKE and SCHWARTZ, 1966) showed no qualitative changes in amino acids but a decline in haemolymph protein. However, apart from the difference in modes of treatment of the two species, there was a difference in the time interval of analysis, the *B. germanica* larvae being assayed after 8 days on dietary glucose-¹⁴C (HENRY, 1962), whereas the *P. americana* larvae were assayed 69 to 129 days after lysozyme injection (MALKE and SCHWARTZ, 1966).

We have shown (WHARTON and LOLA, 1969a, b) that lysozyme induces large quantitative changes in most of the amino acids of the blood, in blood volume, in the Na⁺/K⁺ ratio, and in certain other characteristics. A blood volume increase with increased flow rate and clotting time was also shown to occur upon reinjection of lysozyme into adults whose symbionts had been killed and essentially destroyed by a previous injection of lysozyme. These effects were therefore considered to have been induced probably not by an indirect effect of lysozyme from the release of toxic bacterial products, but rather by a direct effect of lysozyme retained for a considerable period in the insect (WHARTON, 1969).

While these results indicated that lysozyme could change the blood picture in an originally xenic insect without the intervention of symbiont debris, it was wholly unknown what action it might have on aposymbiotic insects that had never harboured the bacterial symbiont. Indeed, little was known of the biochemical make-up of the aposymbiotic *P. americana* and nothing of the blood volume, amino acid, and ionic constitution of its haemolymph. *F*₁ aposymbiotic cockroaches derived from antibiotically treated stock were therefore used with the view of

differentiating the action of lysozyme from any contribution made by the symbiont. Our results will show that lysozyme does alter certain features of the blood picture of the aposymbiotic cockroach. In addition, the aposymbiotic F_1 progeny of antibioticly treated stock are not insects with deficiencies merely, but on the contrary have enhanced qualities that set them apart.

MATERIALS AND METHODS

One- to 3-week-old adult male *Periplaneta americana* were used. The aposymbiotic insects were the F_1 progeny of parents that had been fed continuously as larvae and/or adults on a diet of ground Purina Laboratory Chow containing 1% each of aureomycin, terramycin, and sulphisoxazol. The oöthecae from which the F_1 generation was derived were collected from females that had been reared on the antibiotic diet for not less than 4 months. The F_1 nymphs were typically russet brown in colour, and the adults were conspicuous by the pale cream colour of the ventral abdomen, but otherwise appeared normal and vigorous. Smear preparations (WHARTON, 1968) of the fat body of several of the F_1 larvae and adults failed to show any bacteroids.

The insects were injected with 0.05 ml of a 2% solution of 3 × crystallized, dialysed, and lyophilized egg-white lysozyme (Sigma) in 0.5% NaCl, or with saline alone, or were uninjected (WHARTON and LOLA, 1969a, b), and were bled about 2 weeks later to provide appropriate quantities of blood for analysis. Blood volume was determined (WHARTON *et al.*, 1965; WHARTON and LOLA, 1969a, b) on 20 μ l amounts of blood from each insect. Blood cell counts could be made in most instances with the aid of a Thoma white cell pipette. However, the blood from untreated or saline-injected 'normal' insects usually clotted too readily for its use. To reduce clotting through surface contact, and to permit more rapid mixing, a 50 μ l microsampling pipette (Corning) was used. As soon as the leg was cut, the exuding blood was dusted with finely powdered potassium oxalate and drawn up into the pipette to a mark at 10 μ l. It was then immediately ejected into 0.19 ml of a 0.2% potassium oxalate solution contained in a small test-tube and repeatedly pumped back and forth to disperse the cells. An essentially uniform dispersion resulted, as observed microscopically. Clotting time was determined on a 10 μ l sample of blood which was placed on a glass cover-slip and inverted over a deep-well slide, the lip of which was ringed with vaseline. The preparation was observed microscopically and the progress of coagulation timed.

Inorganic ions were determined with three separate samples of blood, each of which was composed of 100 μ l drawn in 20 μ l aliquots from 5 different insects. The samples were dried, ashed, and analysed in a Perkin Elmer Atomic Absorption Spectrophotometer (WHARTON and LOLA, 1969b).

Amino acids were determined on approximately 1 ml samples of pooled blood which were collected in 0.2% potassium oxalate solution and precipitated with trichloroacetic acid. The supernatant was decanted and repeatedly washed with di-ethyl ether and then dried down under reduced pressure at 35°C. The samples were then analysed in an amino acid analyser (WHARTON and LOLA, 1969b).

RESULTS

Mortality, blood coagulation, and blood volume

The aposymbiotic insects had a lower mortality rate than the symbiotic following lysozyme injection; within 2 weeks the death rates were 6 and 16 per cent respectively. The aposymbiotic insects bled more freely than the xenic and following lysozyme injection bled more freely than saline-injected or uninjected controls, and the blood took longer to clot. As a result, wounded insects that had been treated with lysozyme bled to death more readily than normal, untreated insects. For example, all of 13 F_1 aposymbiotes that had been injected with lysozyme and later bled by disarticulation died within 7 days, as against 6 out of 11 which had been injected with saline and bled. Comparisons of coagulation time and the quality of clot showed that the blood of the lysozyme-treated insects coagulated much more slowly than that of the 'normal', saline-injected insects. Whereas normal blood clotted rapidly and formed tightly contracting masses of coagulum, the blood from lysozyme-treated insects formed small islets of coagulum consisting of few cells which were loosely linked. In normal blood, smaller clots were strongly attracted to a larger mass, whereas it was not unusual for adjacent islets of clot in the lysozyme-treated blood to separate. Coagulocytes with extruded contents often failed to draw adjacent cells to them. Blood from saline-treated aposymbiotic insects also was generally weaker than 'normal' in its tendency to clot, and responded to lysozyme like the 'normal'. The total blood cell count of the lysozyme-treated and the aposymbiotic insects was much reduced (Table 1).

TABLE 1.—TOTAL BLOOD CELL COUNTS/mm³ OF SYMBIOTIC AND APOSYMBIOTIC *P. americana*, UNTREATED OR TREATED WITH LYSOZYME OR SALINE

	Symbiotes			Aposymbiotes		
	Untreated	Injected with saline	Injected with lysozyme	Untreated	Injected with saline	Injected with lysozyme
Average	13,331 (10)	19,486 (10)	6655 (10)	5120 (6)	3948 (10)	5330 (10)
Range	22,264— 7084	32,550— 8400	12,440— 1600	7920— 3520	12,600— 756	11,360— 1428

Ten days post-injection
Number of insects in parentheses.

The average blood volume of 30 per cent in untreated aposymbiotic insects was significantly lower than the average for the symbiotic insect (WHARTON *et al.*, 1965; WHARTON and LOLA, 1969a). After treatment with lysozyme the blood volume was significantly higher than that of saline-injected or uninjected controls (Table 2), and, notwithstanding the lower blood volume, the difference was almost as great as that generally observed between similar groups of symbiont-bearing insects. These results show that lysozyme can act directly on the tissues in the absence of the bacterial symbiont or its products.

Further confirmation was sought by determining whether inactivated lysozyme could elicit a similar response. Heating a solution of lysozyme of acid pH at 100°C for a few minutes destroys some of its activity (JOLLÈS, 1967). When a 2% solution of lysozyme was heated at this temperature for 1.5 hr it was precipitated

TABLE 2—BLOOD VOLUME PER CENT*, AND BLOOD FLOW AFTER PUNCTURE OR DISARTICULATION, IN THE APOSYMBIOTIC MALE AMERICAN COCKROACH 12 days AFTER TREATMENT WITH LYSOZYME

Blood volume (μ l/100 mg)			Blood flow (μ l)	
Lysozyme	Saline	Uninjected	Lysozyme	Saline
39.1 \pm 7.55 (23)	31.7 \pm 3.87 (20)	30.6 \pm 4.4 (11)	56.1 \pm 25.4 (31)	33 \pm 12.1 (28)
$P = < 0.01$			$P = < 0.01$	

Number of insects in parentheses.

* With standard deviations.

and inactivated, that is, the supernatant failed to lyse a suspension of *Micrococcus lysodeikticus* cells. When the entire heated enzyme was macerated and homogenized it formed a dense suspension that could be injected satisfactorily through a 27-gauge needle. Normal symbiont-bearing males were injected with a heated or unheated preparation of 2% lysozyme, or with saline alone, and assayed for blood volume 17 days later (Table 3). The unheated, active enzyme induced a statisti-

TABLE 3—EFFECT OF HEAT-ACTIVATED LYSOZYME ON BLOOD VOLUME OF NORMAL COCKROACH

Blood volume (μ l/100 mg)		
Unheated lysozyme	Heated lysozyme	Saline
35.8 \pm 6.6 (13)	30.8 \pm 2.9 (8)	25.7 \pm 4.5 (12)
$P = 0.05$		$P \sim 0.05$

Number of insects in parentheses.

cally significant increase in blood volume, and an observed increase in blood flow and coagulation time, and a decrease in cellularity. On the other hand, the heated, inactivated enzyme induced an increase in blood volume, but one that was significantly less than that induced by the unheated enzyme. We do not know what influence, if any, the physical differences in the two enzyme preparations might have had in producing the observed effects or if the activity of the heated enzyme was recovered in the insect, but it is evident that the response to the active enzyme was not simply that to a non-specific foreign body. In view of the fact that active lysozyme induced these effects in the aposymbiotic (Table 2) as well as the xenic

insect (WHARTON and LOLA, 1969a), the conclusion seems warranted that the observed effects were due to or were initiated by the enzymic action of lysozyme on the tissues.

Amino acids

Table 4 shows the concentration and content of amino acids in the blood of saline-injected symbiont-bearing and aposymbiotic male cockroaches. The aposymbiotic insect was found to have three times the average content of amino acids as the normal, with individual differences amounting to almost eight-fold.

TABLE 4—AMINO ACID CONTENT (μ moles per insect and ratios) OF BLOOD OF NORMAL AND APOSYMBIOTIC COCKROACHES BEFORE AND AFTER TREATMENT WITH LYSOZYME

	Normal cockroaches			
	Saline (μ moles)	Ratio: Lysozyme Saline	Ratio: Aposymbiotic (saline) Normal (saline)	Ratio: Aposymbiotic (lysozyme) Aposymbiotic (saline)
Lysine	0.022	3.90	2.50	2.13
Histidine	0.05	2.00	2.44	1.35
Arginine	0.017	2.24	2.09	1.57
Aspartic acid	0.003	0.95	1.26	0.75
Threonine	0.129	1.75	2.60	1.20
Serine	0.024	1.91	2.61	1.60
Glutamic acid	0.020	2.40	1.10	0.87
Proline	0.161	1.61	3.09	1.32
Glycine	0.167	1.94	3.48	1.17
Alanine	0.018	1.80	6.48	1.10
Valine	0.028	1.64	5.47	1.00
Methionine	0.010	0.85	0.81	1.06
Isoleucine	0.009	2.96	7.90	1.00
Leucine	0.017	2.90	7.10	0.83
Tyrosine	0.056	1.25	0.41	1.06
Phenylalanine	0.014	0.46	0.83	0.73
Homoserine	—	—	—	0.79
H-serine lactone	—	—	—	1.00

The greatest increases were in isoleucine, leucine, valine, and alanine. Tyrosine was reduced by almost 60 per cent, while phenylalanine and methionine were only slightly so. Glutamic and aspartic acids were only slightly changed. A further notable difference was the appearance in relatively high concentration of the amino acid homoserine. This amino acid had not been seen before even in those insects whose symbionts has been destroyed by lysozyme.

Table 4 also shows the effect of lysozyme on the amino acid content of the aposymbiotic insect. Increases occurred in lysine, histidine, arginine, serine, and

possibly proline. The differences were fewer and generally less pronounced than those that occurred in the symbiont-bearing insect. Nevertheless, comparison of molar ratios between different groups of amino acids in lysozyme-treated normal and aposymbiotic insects reveals widely varied relationships rather than a consistent pattern (Table 5).

TABLE 5—MOLAR RATIOS OF AMINO ACIDS IN BLOOD OF NORMAL AND APOSYMBIOTIC *P. americana* BEFORE AND AFTER TREATMENT WITH LYSOZYME*

Amino acids	Normal cockroach			Aposymbiotic cockroach	
	Uninjected†	Saline injected	Lysozyme injected	Saline injected	Lysozyme injected
Val/Met	2.56	2.98	5.42	19.0	17.2
Gly/Arg	10.5	9.55	8.5	16.5	11.9
Hia/Leu	2.46	3.12	2.82	1.19	1.64
Ala/His	1.25	0.33	0.29	0.84	0.81
Ala + Gly/Asp	1.3-1.5†	65	124	183	288
Glu/Ala	1.3-1.5†	1.12	1.46	0.18	0.15
Thr/Asp	0.5-0.5†	43	81	84	144

* With molar ratios of amino acids in sera of dog, rat, and man (SCHULTZ, 1964).

† Calculated from data of KNORRE (1967, Table 2) on blood of *P. americana* adults.

Inorganic ions

The Na^+/K^+ ratio in the blood of symbiotic males was shown to be approximately 8.6, between the first and second week; following the injection of lysozyme it had increased to 12.1 (WHARTON and LOLA, 1969b). By contrast, the Na^+/K^+ ratio in the F_1 aposymbiotic males was almost doubled at 16.3 with an increase in the Na^+ and a decrease in the K^+ concentration; it was little changed by the injection of lysozyme (Table 6). Thus, the two principal osmoregulators, the

TABLE 6— Na^+ AND K^+ CONCENTRATION (ppm) IN THE BLOOD OF THE APOSYMBIOTIC COCKROACH 11 days AFTER INJECTION OF 0.05 ml OF 2% LYSOZYME IN 0.5% NaCl SOLUTION

	Na^+	K^+	Na^+/K^+
Lysozyme	3036	203	15.0
Saline control	2924	179	16.3

amino acids and the Na^+ , were greatly increased in concentration in the aposymbiotic insects. Whereas significant differences in amino acid concentrations and ratios resulted from the administration of lysozyme, the Na^+/K^+ ratio was apparently not altered as it was in the symbiotic cockroach.

DISCUSSION

The results show that injection of lysozyme in the aposymbiotic cockroach produced an increase in blood volume, blood flow, and clotting time that is comparable to the effect produced in the xenic cockroach. The weak coagulability of the blood of the lysozyme-injected insects is presumably correlated with a reduction in protein (WHARTON and LOLA, 1969b). Lysozyme also caused an increase in lysine, histidine, arginine, serine, and proline, with the greatest increase occurring in lysine, as in the xenic insect, and changed the ratios of several amino acids to one another. It is thus evident that lysozyme is capable of injuring the symbiont-free insect.

It is remarkable that lysozyme should have these similar effects in animals that differ in important respects, whose osmoregulatory systems have been altered, with changed Na^+/K^+ ratios and large differences in amino acid ratios and content that point to changed protein conditions. The effect of lysozyme in the symbiont-free insect differed in degree, however, from its action in the symbiotic insect. Whereas 12 out of 16 amino acids in the symbiotic insect increased from 50 to 300 per cent, only the above named 5 amino acids of the aposymbiotic insect increased, and the range was from approximately 30 to 110 per cent. Moreover, the aposymbiotic insects have a lower mortality rate than the symbiotic following lysozyme injection, and there is no histologic evidence of injury in the testes as has been observed in the symbiont-bearing insect. This suggests that the aposymbiotic insect is more resistant to lysozyme, and/or that some of the toxic effect following lysozyme injection in the symbiotic cockroach may have been accentuated by products of bacterial disintegration from the mycetocytes.

The molar ratios of amino acids from the blood of our saline-injected normal cockroaches compare favourably with those of KNORRE (1967), except for a lower alanine/histidine ratio in our samples. We do not know which is the more precise, but would point out that saline injection did reduce the alanine slightly to 0.78 of the uninjected (WHARTON and LOLA, 1969b), and Knorre's technique of obtaining blood directly from the heart may have avoided entraining histidine from the site of puncture as may have occurred in our procedure. Both sets of data, however, differ greatly from the molar ratios of mammalian sera (SCHULTZ, 1964), and also contrast with the findings of HENRY *et al.* (1964) that the molar ratios of the amino acids from the whole animal protein hydrolysates of the German cockroach were similar to those of vertebrates and other invertebrates, although histidine, lysine, tyrosine, leucine, isoleucine, valine, alanine, and cystine were less abundant.

The very high amino acid content of the aposymbiotic cockroach—as here obtained by treatment with antibiotics—would seem to fulfil more than an osmoregulatory function, although a Na^+/K^+ ratio almost twice as high in the aposymbiotic as in the symbiotic cockroach indicates a strong shift. It may be pathognomonic of the growth and reproductive aberrations. Or it may, on the contrary, be of survival value, functioning to maintain a high level of proteins necessary in defence of the host against infection. This could account for the relatively weaker effect of lysozyme with respect both to its induced mortality and

evoked amino acid response. The great differences in quantity, ratio, and kind of amino acids are evidence that the F_1 aposymbiotic cockroach obtained by antibiotic treatment of the parent stock is metabolically and physiologically a very different animal from the symbiont-bearing type. The indicated differences are large and some, perhaps, are referable to the bacterial symbiont inasmuch as they also appear in cockroaches made aposymbiotic by other means. A case in point is the lack of melanization, which is a common result of the procedures used up to now. HENRY and BLOCK (1960, 1961) found that the haemolymph of aposymbiotic *B. germanica* larvae derived from aureomycin-treated parents lacked 6 amino acids—tyrosine, phenylalanine, isoleucine, valine, arginine, and probably threonine, and concluded that the symbionts played an important rôle in the synthesis of essential and non-essential amino acids. They attributed the conversion of inorganic sulphur to methionine to the intracellular bacteria. Our results are quite divergent, marked by a twofold overall increase in the amino acids, with eight times the normal amount of isoleucine, 5.5 times the normal amount of valine, twice as much arginine and 2.5 times as much threonine. Tyrosine was reduced to 41 per cent of normal, but phenylalanine was not greatly reduced. The methionine level was well maintained (80 per cent), and serine increased by 160 per cent. If these differences in amino acid concentrations are due to the loss of the bacterial symbionts, it means that the bacteria perform different metabolic functions in the two species of cockroach and are physiologically distinct.

Homoserine is a source of methionine, threonine, and isoleucine in microorganisms (DAVIS, 1955). High concentrations of homoserine in the aposymbiotes might therefore suggest a significant change in the metabolism of the sulphur amino acids. However, in neither the normal nor the aposymbiotic insect did lysozyme affect the methionine concentration significantly. On the other hand, lysozyme increased the concentration of threonine and isoleucine when injected into the normal insect, but not the concentration of isoleucine and only slightly that of threonine in the aposymbiotic. The aposymbiotic insects showed great differences in concentration of these amino acids, and it is remarkable that the methionine concentration seems to have been so little affected either by lysozyme injection or the aposymbiotic state. It is in the aposymbiotic insect that homoserine makes its appearance, and although the methionine concentration is somewhat reduced in the aposymbiotic insect, it could hardly be considered to have been blocked to a sufficient degree to explain the high concentration of homoserine. The increase in homoserine may rather be the source of the increase in isoleucine and threonine.

It is a moot question whether any of the observed changes are due to the absence of the bacterial symbiont or/and to the toxic action of the sterilant. The lack of pigmentation observed in the progeny of all sterilized insects, regardless of the mode of sterilizing, is caused by a seemingly specific process which has been brought about by the absence of the symbiont. Thus, the relative depletion of tyrosine and the somewhat low level of phenylalanine might be a cause or aspect of melanin deficiency. However, the process of melanization is complex and not

uniform (HENDERSON *et al.*, 1962). HENRY (1962) finds the failure of aposymbiotic *B. germanica* to synthesize tyrosine 'particularly interesting since this amino acid is essential to the tanning of insect cuticle'. The complexity of the problem is magnified by the relative lack of vitamins, specifically ascorbic (GALLAGHER, 1962; PIERRE, 1964) and pantothenic (GALLAGHER, 1962) acids, which are activators of tyrosinase. MALKE and SCHWARTZ (1966), however, could find no vitamin shortage.

The generally high level of the amino acids in the aposymbiotic insect appears to favour a highly competent, vigorous organism. On the other hand, the obvious growth and reproductive defects indicate functional deficiencies which oppose this view. Differences of 100 or 200 per cent in certain amino acids of the blood occur at different stages of larval development in the cockroach (KNORRE, 1967), and in *Bombyx* there may be manifold increases in the 'sericigenous' amino acids and still greater increases in methionine (FLORKIN and JEUNIAUX, 1964), but these differences are normal to physiological development. In adult symbiotic and aposymbiotic cockroaches, on the other hand, the different physiological states are presumably essentially stable and therefore set the aposymbiote apart as an organism *sui generis*. However, to discover the essential properties of the cockroach it is not enough merely to separate the insect from its symbiont. Questions remain, as to the effect of the sterilizing procedure on the insect, and the probable contribution of the bacteroids. The latter can only be answered with some degree of certainty when the bacteroids have been cultivated and their physiology assessed, and this has not yet been accomplished (BROOKS and RICHARDS, 1966).

The changes induced by lysozyme in the aposymbiotic insect tend to confirm the validity of the suggestion put forward in our earlier paper (WHARTON and LOLA, 1969a) on the symbiotic cockroach, that lysozyme probably plays a significant rôle in the inflammatory process. That it should have a generally similar action in the aposymbiotic insect, which differs markedly from the 'normal' in important respects, attests all the more to the likelihood of such a rôle.

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