antibodies only one segregant was found. This was susceptible to chloramphenicol and streptomycin but was resistant to tetracycline and kanamycin.

Discussion. It is impossible to give a precise rate for the transfer of the R factor but clearly it was able to spread epidemically and, eventually, infect a high proportion of K12. This occurred without the selective pressure of antibiotic treatment. However, the rate of spread was much slower than *in vitro*. Eighteen hours after feeding AK to the mice, only 0.005% of K12 had acquired antibiotic resistance. *In vitro* 1.6% acquired drug resistance in 18 hours. Only after more than 2 weeks did more than 1% of the K12 in the gut acquire the R factor. Nonetheless, the rate of acquisition of the R factor was much higher than the rate of segregation.

It is uncertain to what extent we may extrapolate these findings to conventional animals. There are many differences between germ-free and conventional animals. We cannot be certain that observation in mice contaminated only with *Enterobacteriaceae* would be similar in conventional mice who are contaminated with many species of bacteria. For example, the bacterial density of *Enterobacteriaceae* at the sites in the gut where the milieu is suitable for R factor transfer may be much different in mono-contaminated germ-free mice and in conventional animals. Also, subtle differences in the physical and chemical makeup of intestinal contents could profoundly affect the efficiency of R factor transfer. *In vitro*, R factor transfer is greatly inhibited by anaerobic growth and by inhibitors of oxidative phosphorylation (6). If the intestinal contents of mono-contaminated germ-free mice were less anaerobic than the gut contents of conventional mice, the rate of transfer of the R factor would, in all probability, be greater. Further work is required to explore these factors.

Summary. An antibiotic resistant *Klebsiella pneumoniae* containing an R factor was fed to germ-free mice previously colonized with an antibiotic susceptible *E. coli*. Within a month, 21% of the *E. coli* acquired multiple drug resistance without the selective pressure of antibiotic therapy. However, the rate of spread of the R factor was significantly slower than *in vitro*.

We thank Miss Dorinne Kan for her able assistance.

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Received Dec. 21, 1967. P.S.E.B.M., 1968, Vol. 128.

An Induced Bactericidin in the Spiny Lobster, *Panulirus argus** (33021)

E. EDWARD EVANS, BARBARA PAINTER, MARJORIE L. EVANS,
PETER WEINHEIMER[1] AND RONALD T. ACTON[1]
University of Alabama Medical Center, Birmingham, Alabama 35233

Studies on the evolution of immunity (1) have shown that processes long recognized in mammals are characteristic of most vertebrates. One aspect of vertebrate immunity not yet convincingly demonstrated among the invertebrates is the synthesis of specific immu-

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* Supported by USPHS Grant AI-02693. A portion of the work was done at the Lerner Marine Laboratory, American Museum of Natural History under support of the Office of Naval Research, ONR-552-07.

[1] Supported by USPHS Training Grant AI-00293.

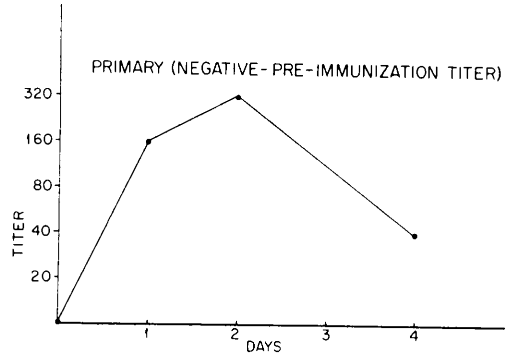

FIG. 1. Primary response curve of hemolymph bactericidin following immunization with gram-negative bacillus, EMB-1.

noglobulins; however, both humoral and cellular mechanisms of immunity have been reported among invertebrates (2–7).

From consideration of the primary structure of heavy and light polypeptide chains of vertebrate immunoglobulins, several investigators (8–10) have postulated that the genes coding for these proteins have evolved from a common ancestor. A model has been proposed for the genetic origin of immunoglobulins based on a precursor L chain with a molecular weight of about 12,000 (11).

Although immunoglobulin evolution may have involved only the vertebrates, it is our contention that the capability for antigenic recognition and for synthesis of primordial immunoglobulins may have first appeared among the invertebrates.

One approach toward inducing possible immunoglobulin synthesis has been to immunize invertebrates with bacteria isolated from their normal gut flora in the expectation that the animal through repeated experience may have learned to respond to such an antigen; whereas, a protein such as bovine albumin might lie beyond the animal's capability. The West Indian spiny lobster, *Panulirus argus*, has been shown capable of synthesizing a hemolymph bactericidin induced by injections of bacteria.

Methods. Live, mature specimens of *P. argus* were maintained in sea water at the Lerner Marine Laboratory, Bimini, Bahamas. The animals were immunized by intracardial (pericardial sinus) injections of 10^9 cells of various bacteria in 0.9% sodium chloride solution. Various bacterial strains to be used as antigens were isolated from the lower gut by plating on EMB agar and blood agar plates. Brain heart infusion broth or agar was used for subsequent cultures. The most effective antigen was strain EMB-1, a gram-negative enteric bacillus. In some cases, live bacteria were used; in others, the bacteria were killed with 0.5% (v/v) formalin. Animals were bled from the pericardial sinus before immunization ("0 hours") and at various times postimmunization. The bactericidal assay procedure of Schwab and Reeves (12) was used. Complement-inactivation procedures have been previously described (13, 14).

Results. Figure 1 shows a typical primary response of a spiny lobster to an intracardial injection of killed suspensions of the gram-negative rod, EMB-1. The bactericidal titer of the hemolymph usually reached a peak at 36–48 hours although in a few instances the maximum titer was first seen at 24 hours. Beyond 48 hours, a slow decline was the rule with some activity occasionally demonstrable at the end of 2 weeks. Live cultures or killed vaccines (10^9) were equally effective in inducing this bactericidin.

The hemolymph of some animals displayed a low level of activity before immunization. An example of this type is seen in Fig. 2. The maximum titer reached by different animals ranged from 40 to 320. Active sera failed to agglutinate cells in a standard tube agglutination test.

To check the specificity of the bactericidal

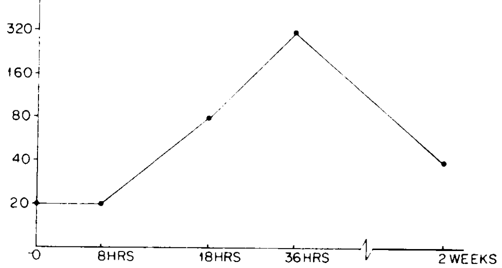

FIG. 2. Bactericidal response in animal with preexisting titer.

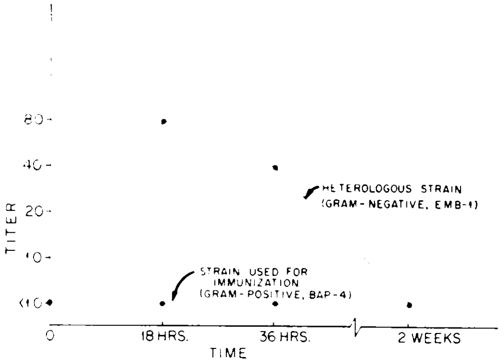

FIG. 3. Nonspecific response induced by gram-positive bacillus, BAP-4.

response, lobsters were immunized and assayed with a variety of other bacteria. A gram-positive rod, BAP-4, isolated from the gut of this lobster induced a peculiar nonspecific response (Fig. 3). Animals immunized with formalin-killed BAP-4 displayed no bactericidal activity for the homologous strain, but bactericidal activity against EMB-1 was present, reaching a peak at 18 hours and declining to preimmunization levels by 2 weeks.

Immunization of animals with the EMB-1 gram-negative rod often resulted in the development of bactericidal activity for *S. typhosa* as well as for the immunizing culture. Table I shows such an experiment. The titers for *S. typhosa* were lower than for the homologous strain at every bleeding tested. Table II presents data from animals immunized with H antigen of *S. typhosa*. In two animals the heterologous, EMB-1 titer was higher than the homologous at its peak. In the third animal, the two titers were the same. Reciprocal immunization of rabbits with EMB-1 and *S. typhosa* revealed no cross-reaction between the two.

TABLE I. Bactericidal Titers of *P. argus* Immunized with EMB-1 Vaccine.

	Assay culture[a]	
Time of bleeding	Immunizing strain[b]	*S. typhosa*
Preimmunization	20	<10
8 hours	20	10
18 hours	320	80
36 hours	320	80
2 weeks	40	<10

[a] Titers shown are reciprocals of hemolymph dilution.

[b] Formalin-killed vaccine.

TABLE II. Bactericidal Titers of *P. argus* Immunized with *S. typhosa* (H) Vaccine.

		Assay culture	
Animal no.	Day	EMB-1	*S. typhosa*
20–6	0[a]	<10	<10
	2	160	40
20–10	0	<10	<10
	2	40	<10
20–12	0	<10	<10
	3	80	80

[a] Preimmunization.

TABLE III. Specificity of *P. argus* Bactericidin.

		Animal no.[a]	
Gram reaction	Assay culture	IPN-28 173	IPN-29 183
Negative	*P. aeruginosa*	<10	<10
	E. coli	<10	10
	EMB-1	40	160
Positive	*S. aureus*	<10	<10
	B. megaterium	<10	<10
	B. subtilis	<10	<10

[a] Reciprocal of bactericidin titer.

Hemolymph samples from two lobsters immunized with EMB-1 were also tested against two other gram-negative rods, *Pseudomonas aeruginosa* and *Escherichia coli*, (Table III) and three gram-positive bacteria, *Staphylococcus aureus*, *Bacillus megaterium*, and *B. subtilis*.

One of the sera had slight activity against *E. coli* but this was the only cross-reaction noted. Injections of B. H. I. infusion broth produced no response. Injections of 0.9% sodium chloride produced only a slight response in some animals and no response in others. Thus, the bactericidal response was not completely nonspecific. It should be noted that in no case was bactericidal activity obtained for gram-positive bacteria. This may in-

TABLE IV. Inactivation of Bactericidin by Heat (20 min).

	Titer	
Temperature (°C)	IPN-28 175	IPN-28 176
Control (in ice bath)	160	80
50	80	80
55	80	80
60	20	40
65	<10	<10
70	Coagulated	
65 plus unheated hemolymph[a]	<10	

[a] Equal volumes were mixed together.

dicate that this group of bacteria is not affected by the bactericidin described here.

To test the heat stability of this induced bactericidal system, samples of hemolymph were exposed to various constant temperatures for 20 min. Results, shown in Table IV, indicate some diminution of activity at 60°C and complete inactivation at 65°C. Activity could not be restored with unheated normal serum.

The bactericidin was not appreciably diminished by treatment with EDTA or carrageenin (13, 14). The bactericidin also resisted freezing at —25°C and dialysis against 0.9% sodium chloride solution for 7 days. This latter observation suggests a large molecule and this is reinforced by separations on Sephadex G-200 columns to be published elsewhere. The bactericidin could be adsorbed with EMB-1, but antigenically unrelated bacteria were also effective.

Discussion. The hemolymph bactericidin of *Panulirus argus* was readily induced by killed or living suspensions (10^9) of culture EMB-1, a gram-negative rod isolated from the gut of this lobster. Other bacteria such as the antigenically unrelated *S. typhosa* were also effective inducers. A gram-positive rod, BAP-4 induced a completely nonspecific response.

Thus the bactericidin resembles immunoglobulin in that it can be induced by antigen and in its molecular size; however, it lacks the degree of specificity ordinarily associated with mammalian antibodies and does not agglutinate antigen. It is possible, of course, that relative nonspecificity may be a characteristic of primordial immunoglobulins.

The bactericidin is unlike complement-dependent bactericidal systems (15) in its relative heat stability and its failure to be inactivated by reagents that inactivate vertebrate complement (13, 14). After heat-inactivation at 65°C, the addition of unheated normal hemolymph failed to restore activity. The possibility that this substance represents a primordial immunoglobulin is under continuing investigation.

Summary. Spiny lobsters, *P. argus*, immunized with living or killed bacteria, have been shown to synthesize a nondialyzable bactericidin. This substance was present in the hemolymph and was usually detectable within 12 hours. In the primary response, it reached a peak within 24–48 hours. The most effective antigen was a gram-negative bacillus, EMB-1, isolated from the normal intestinal flora of healthy *P. argus*. Varying results were obtained when specificity was tested with heterologous bacteria, but the bactericidin was less specific than mammalian antibody. Heating at 65°C for 20 min was required for complete inactivation. EDTA, dialysis, carrageenin, and freezing failed to inactivate this substance. It could be adsorbed by homologous and heterologous bacteria.

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Received Jan. 25, 1968. P.S.E.B.M., 1968, Vol. 128.

In Vivo Lipogenesis in the Domestic Chicken (33022)

GILBERT A. LEVEILLE, EUGENE KEVIN O'HEA, AND KRISHNA CHAKRABARTY
Division of Nutritional Biochemistry, Department of Animal Science, University of Illinois, Urbana, Illinois 61801

The metabolism of mammalian adipose tissue has received considerable attention in recent years. Numerous studies have demonstrated that rat adipose tissue can synthesize large quantities of fatty acids *in vitro* (1). In fact, adipose tissue appears to be the major site of fatty acid synthesis in the mouse (2) and rat (3,4). In contrast to studies with mammalian species, avian adipose tissue has received little attention. Goodridge and Ball reported that pigeon adipose tissue had a very low lipogenic capacity (5) and similar results have been obtained with chicken adipose tissue (6). *In vivo* studies have led to the conclusion that pigeon adipose tissue accounts for only about 4% of total fatty acid synthesis, while liver is responsible for the remainder (7). In view of the low *in vivo* lipogenic capacity of chicken adipose tissue (6), it might be anticipated that in this species, as reported for the pigeon, liver is the major site of fatty acid synthesis. The studies reported were undertaken to evaluate the relative importance of liver and adipose tissue as sites of fatty acid synthesis in the chicken.

Methods. Male crossbred chicks (New Hampshire ♂ × Columbian ♀) weighiing 490–630 gm were used. The chicks were fed a semipurified diet similar in composition to that described earlier (8). On the day of experiment the chicks were given glucose-U-^{14}C or acetate-1-^{14}C intraperitoneally in 1 ml of saline. Thirty and 60 min after administration of the tracer, blood was obtained by cardiac puncture with a heparinized syringe. The animals were then killed by cervical dislocation and the liver and adipose tissues were rapidly removed and cooled on ice. Adipose tissue was obtained from the neck region. Lipid was extracted with chloroform: methanol (2:1, v/v) as previously described (9). A portion of the lipid extract was taken for liquid scintillation counting. The remainder of the lipid extract was evaporated to dryness and saponified by refluxing with 10 ml of methanolic KOH (5%). Following saponification the solution was diluted with 10 ml H_2O and the nonsaponifiable lipids were removed with 3 successive 5-ml portions of petroleum ether (bp 30–60°). The fatty acids were removed in a similar manner after acidification of the aqueous fraction with concentrated HCl. The samples were prepared for liquid scintillation counting by evaporating to dryness and dissolving the lipids (total lipids, nonsaponifiable lipids, or fatty acids) in 10 ml of a toluene scintillant (6).

Results. The results of two experiments designed to study the ability of the chick to utilize glucose for lipid synthesis are presented in Table I. The results of these two experiments are in agreement and show that the chick can readily incorporate glucose carbon into lipids. The amount of radioactivity in fatty acids was higher in liver than in adipose tissue at both time periods studied. However, during the first 30 min the incorporation of glucose into total lipids was greater in adipose tissue than in liver but only 2–4% of this radioactivity could be accounted for in fatty acids and nonsaponifiable