BIOCHEMICAL CHANGES IN MOUSE BLOOD AND LIVER FOLLOWING *STAPHYLOCOCCUS AUREUS* INFECTION

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STAPHYLOCOCCUS AUREUS causes many serious and minor infections including septicemia, pneumonia, boils, carbuncles, impetigo and others. Careful diagnosis and systematic treatment are necessary to avoid complications.[1] Recently it has been suggested that "chemotherapy", or perhaps better "chemical therapy", in combination with "antibiotic treatment" might have promise. Additionally, to avoid empirical (instead of rational) use of antibiotics, a thorough knowledge of the altered metabolism of infection is necessary.

In view of our past experiences[2-4] we have designed an experimental model in mice where we inject *Staphylococcus aureus*, Smith (obtained from a patient with osteomyelitis and maintained in the laboratory) by *in vitro* passage.[3] A heavy dose of 1×10^9 cells is injected by the intraperitoneal route into white Swiss male mice weighing approximately 22 gm. This infection produces death in 254 minutes ($\pm$ 27·7 minutes). In the present paper, autopsies were done at the 'time of death' (in a group of ten mice, when the second animal died the remaining eight were decapitated and used for analysis).

The behaviour of the animal after injection is quite consistent. Piloerection around the neck occurs within 60 minutes. Mice begin grouping together after 90 minutes, presumably due to body cooling. Their temperature falls 20 to 30° F. Unsteady walking begins after 120 minutes followed by dyspnea in about 150 minutes. A fall of the respiratory rate (240/min. to 140/min.) is obvious and respiration later becomes labored and irregular. After 150 minutes the mice begin erratic jumping at irregular intervals and thrash their tails. Death ensues at an average of 254 minutes after infection with a generalized clonic convulsion and incontinuance of urine.

Of the various biochemical analyses done so far, including inorganic salts, sugars, phosphates and nitrogen fractions, a significant variation from the normal (Table I) mice is seen only in case of those analyses which are shown in Table II.

A fall of glucose demonstrates the effect of staphylococcus on carbohydrate metabolism. Subcutaneous injection of glucose within an hour before or after staphylococcal injection increased the survival time 27%. Changes in cholesterol suggest that fat breakdown follows infection. A rise of inorganic phosphate with a concomitant fall of organic phosphate cause a striking rise of inorganic phosphate/organic phosphate (Pi/Po) ratio. This strongly suggests the rapid breakdown of organic phosphate following bacterial infection. The significant

TABLE I

*Normal values of blood and liver of mice for the biochemical analyses which have shown significant changes**

	Whole blood	Liver	References to methods followed
Glucose ..	52·7	125·0	5
Cholesterol ..	139·0	33·0	5
Alkaline phosphatase (s.u.)	4·1	37·4	6
Inorganic phosphate ..	3·8	35·0	7
Organic phosphate ..	42·2	132·3	7
Acid-soluble organic phosphate	N.D.	32·2	7
Inorganic P/Organic P (ratio)	0·09	0·26	7
Non-protein nitrogen ..	13·0	220·0	5
Urea nitrogen ..	8·3	180·0	5
Amino acid nitrogen ..	4·0	35·0	8, 9

* Unless otherwise stated, blood values are in mgm./100 ml. and liver values are in mgm./100 mgm. fresh weight.
N.D. = Not done.

TABLE II

Significant biochemical changes in blood and liver of staphylococcus (S. aureus) *infected mice*

(All values expressed as per cent. normal)

	At death from infection	
	Whole blood	Liver
Glucose ..	67†	12†
Cholesterol ..	7·†	94
Alkaline phosphatase ..	138†	118*
Inorganic phosphate ..	132†	140†
Organic phosphate ..	84*	93
Acid-soluble organic phosphate ..	N D	53†
Inorganic phosphate/organic phosphate	164†	172†
Non-protein nitrogen ..	122*	265†
Urea nitrogen ..	122*	300†
Amino-acid nitrogen ..	125*	115*

* Significant at 10% level. † Significant at 5% level.
N.D. = Not done.

fall of the acid-soluble organic phosphates suggests that energy-rich phosphates and other easily available organic phosphates are disappearing. A high degree of protein breakdown is also clearly demonstrated from the nitrogen analyses. Infection causes the degradation of protein and thus increases the amino nitrogen values which in turn leads by degradation to increased urea formation.

The results are preliminary in nature. The details of the altered biochemical pathways in infected animals are still in the realm of conjecture. The biochemical data reported here could be directly or indirectly related to staphylococcal infection or may even be effects of any death process. The multiple effects of the bacterial toxins make the approach all the more complicated. *In vitro* attempts with mouse liver and staphylococci to attempt to clarify the events have been discouraging. Isolation and identification of the lethal factor will help us approach the problem more systematically. Recently we have been able to extract the lethal factor from the staphylococcus after subjecting the bacteria to high pressure (40,000 psi).[10] Further purification is necessary before it is identified. Until then, more biochemical data need to be gathered from *in vivo* studies. In our experimental model the presence of live bacteria or live cytoplasm is necessary for the fatal effect on the mice. Culture filtrates, penicillin or heat-killed bacteria do not carry the lethal factor. Injection of infected homogenized liver killed normal mice in about the same time (254 minutes) only when the lethal concentration of 1×10^9 bacterial cells/ml. was present in the homogenate. Treatments with hexoses, pentoses, cortisone, phosphate buffer and 1/6 M sodium lactate or 10% oxygen at 6/10 atmospheres are found to be effective in increasing the survival time.[11-14] Cortisone or oxygen treatment can lead to 40–95% prolongation in the length of life of infected mice. Work is still under progress to establish the pathway of staphylococcal effect on carbohydrate, phosphate and nitrogen metabolism.

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GEOLOGY OF MARION ISLAND

MARION Island lies in latitude 46° 49′–46° 59′ S., longitude 37° 35°–37° 55′ E. It measures 12 miles east-west, by 7 miles north-south, and rises to a maximum height of 3890 ft. The island is wholly volcanic. A younger and an older lava series are present, with intercalated agglomerate and numerous large scoria mounds. Most of the island is built up of the older lavas, chiefly dark-coloured olivine basalts varying in their degree of compaction and often carrying phenocrysts of olivine. There are several light-coloured flows with a distinctive platy habit. The platy lavas have a relatively even grain and contain less basic plagioclase. They are trachytes.

The most distinctive feature of Marion Island from off-shore is the very numerous reddish scoria mounds dotted all over the Island. They rise up to 700 ft. above their surroundings. Their conical shape is frequently perfectly preserved, and some retain crater-shaped depressions at their summits.

The age of the older lavas is uncertain. Behind the shifted coast-line the lower slopes of the Island locally exhibit a mature land surface. By analogy with other oceanic islands, they may date back as far as the Tertiary. The later lavas and scoria mounds are clearly of very recent age.—(*Nature*, 1965, 205, 64.)