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*THE VIRULENCE OF BIOCHEMICAL MUTANTS OF KLEBSIELLA PNEUMONIAE**

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Virulence may be defined as an expression of a host-parasite relationship.[1] This definition is essentially ecological. The result of an invasion of the host by a parasite depends upon the efficiency of the host's defensive mechanisms relative to the ability of the parasite to proliferate and establish itself. If the parasite does not obtain needed nutrilites from the host, it cannot proliferate. Bacon, Burrows and Yates [2–4] have reported that the decreased virulence of purine-requiring mutants of *Salmonella typhosa* inoculated into the peritoneal cavity of the mouse was due to the absence of purine in the cavity. This observation suggested a procedure for "assaying" specific areas of the host for the presence of amino acids, pyrimidines and purines by means of biochemical mutants of a pathogen. The results of titrations of the virulence of biochemical mutants of *Klebsiella pneumoniae* indicate that such a procedure may be feasible.

Materials and Methods.—Mutants of *K. pneumoniae* (strain Kp, Type B) were obtained by means of ultra-violet irradiation followed by penicillin treatment.[5, 6] The M-9 medium[7] was used whenever a synthetic medium was required; Noble agar (Difco) was added to a concentration of 1.5% to prepare solid media. Nutritional requirements were determined auxanographically.

Strain Kp is virulent for the Namru strain mice[8] when inoculated either

intraperitoneally (LD-50 = *ca.* 20 cells) or intranasally (LD-50 = *ca.* 8×10^3 cells). The Namru strain of mice was used throughout. The virulence of the biochemical mutants was determined by inoculating mice weighing approximately 20 g. intraperitoneally with 0.2 ml. or intranasally with 0.05 ml. of a saline suspension of thoroughly washed cells. Aliquots

TABLE 1

RELATION BETWEEN VIRULENCE AND NUTRITIONAL REQUIREMENTS OF BIOCHEMICAL MUTANTS OF *Klebsiella pneumoniae* INOCULATED INTO MICE EITHER INTRAPERITONEALLY OR INTRANASALLY

		ROUTE OF INOCULATION			
		INTRAPERITONEAL		INTRANASAL	
MUTANT	REQUIREMENT	DOSAGE	DEAD/TOTAL	DOSAGE	DEAD/TOTAL
Wild	None	3×10^2	2/5	7×10^3	0/5
		3×10^3	5/5	7×10^4	3/5
		1×10^4	9/10	3×10^5	9/10
		1×10^5	10/10	3×10^6	10/10
34	Threonine	1×10^4	10/10	3×10^5	10/10
		1×10^5	10/10	3×10^6	10/10
2	Tyrosine	1×10^4	9/10	3×10^5	8/10
		1×10^5	10/10	3×10^6	10/10
40	Methionine	1×10^4	9/10	3×10^5	10/10
		1×10^5	10/10	3×10^6	10/10
27	Leucine	1×10^4	9/10	3×10^5	8/10
		1×10^5	10/10	3×10^6	10/10
17	Histidine	1×10^4	10/10	3×10^5	10/10
		1×10^5	10/10	3×10^6	10/10
22	Uracil	3×10^2	1/5	7×10^3	1/5
		3×10^3	4/5	7×10^4	2/5
2711	Guanine	3×10^2	0/10	8×10^3	0/10
		3×10^3	0/10	8×10^4	0/10
		3×10^4	0/10	8×10^5	0/10
58	Adenine	3×10^2	0/5	7×10^3	0/5
		3×10^3	0/5	7×10^4	0/5
		1×10^4	0/10	3×10^5	0/10
		1×10^5	0/10	3×10^6	0/10
58-01	None	1×10^2	8/10	3×10^3	4/10
		1×10^3	10/10	3×10^4	7/10
		1×10^4	9/10	3×10^5	9/10
		1×10^5	10/10	3×10^6	10/10
58-02	None	1×10^2	2/10	3×10^3	1/10
		1×10^3	3/10	3×10^4	0/10
		1×10^4	6/10	3×10^5	9/10
		1×10^5	9/10	3×10^6	9/10

of a single bacterial suspension were used for intraperitoneal and intranasal inoculations; viable cell counts were made by plating suitable dilutions of the suspension on nutrient agar.

Cultures obtained from the peritoneal fluid of animals succumbing after an intraperitoneal inoculation and from lung tissue of animals succumbing

after an intranasal inoculation were tested to determine whether the specific requirement was still present. In all tests, only thoroughly washed cells were used. Animal experiments were terminated 10 days after inoculation, since no deaths occurred after this period.

Results.—Mice were inoculated with the following biochemical mutants: threonine-less, tyrosine-less, methionine-less, leucine-less, and histidine-less (amino-acids), uracil-less (pyrimidine), guanine-less, adenine-less and "complex" purine-less (purines). The parental strain (Kp) was inoculated as a control. The results summarized in table 1 indicate that the amino-acid-requiring mutants and the pyrimidine-requiring mutant were as virulent as the parental strain when inoculated either intraperitoneally or intranasally. In all cases, cultures recovered from the mice were mutants indicating that reversion to wild type had not occurred in the host. The purine requiring mutants, however, were avirulent when inoculated by either route.

TABLE 2

THE EFFECT OF INTRAPERITONEAL INOCULATIONS OF THE "COMPLEX" PURINE-LESS MUTANT, 27139, OF *Klebsiella pneumoniae* AND EITHER HYPOXANTHINE OR A PURINE POOL*

EXPERIMENT	TREATMENT	TOTAL DOSAGE	DEAD/TOTAL
I	27139	1.3×10^5	0/10
	Hypoxanthine	50 mg.	0/10
	27139 plus hypoxanthine	1.3×10^5 50 mg.	3/10
II	27139	8×10^6	0/10
	Purine pool	24 mg.	0/10
	27139 plus purine pool	8×10^6 24 mg.	10/10

* 15 mg. hypoxanthine, 3 mg. xanthine, 3 mg. adenine, 3 mg. guanine.

Small pads of filter paper containing peritoneal fluid from normal mice were added to the surface of pour plates of the biochemical mutants in minimal agar. Peritoneal fluid supplied the requirements for the amino-acid and pyrimidine dependent mutants but not for the purine dependent mutants. The avirulence of purine-requiring mutants of *K. pneumoniae* inoculated intraperitoneally into the mouse parallels the observations of Bacon, *et al.*,[3, 4] with *S. typhosa.*

If the avirulence of the adenine-less mutant was due to its failure to proliferate in an adenine deficient site, reversion to adenine independence would be expected to lead to virulence. Two reversions were obtained in a population of *ca.* 10^{11} viable cells plated in minimal medium. Both reversions, 58-01 and 58-02, were virulent when inoculated into mice either intraperitoneally or intranasally (see table 1).

The "complex" purine-less mutant, 27139, requires either guanine, ade-

nine, xanthine or hypoxanthine for growth. Since the response of the mutant in terms of growth *in vitro* was greater for a pool of these 4 purines compared with the response for any one of the purines, the effect of inoculating graded dosages of hypoxanthine or a pool of the 4 purines into the peritoneal cavity of mice previously inoculated by the same route with mutant 27139 was determined. Suitable controls were used. The results summarized in table 2 indicate that the response to hypoxanthine was less severe than the response to the purine pool. Cultures recovered from the peritoneal cavity and lungs of the dead mice were mutant indicating that reversions to the wild type were not responsible for the response. Moribund mice were sacrificed and only mutant cultures were recovered. No deaths were observed in the control groups.

Discussion.—The loss of virulence of purine-requiring mutants of *S. typhosa* and *K. pneumoniae* reflects the inability of these mutants to grow in the peritoneal cavity of the mouse. Bacon, *et al.*[3, 4] demonstrated in the case of *S. typhosa* that the decreased virulence of purine-requiring mutants was due to the absence (or perhaps unavailability) of purines in the peritoneal cavity of the mouse. These observations suggest that purine-requiring mutants of any pathogen may display decreased virulence when inoculated intraperitoneally into the mouse. That this situation may be extended to the mouse lung is indicated by the results obtained with the purine-requiring mutants of *K. pneumoniae.*

Since the addition of the specific requirement, purine, permitted the "complex" purine-less mutant, 27139, to proliferate in the host, resulting in the death of the host, it is reasonable to assume that the avirulence of the mutant had been due to the absence or unavailability of purine in the peritoneal cavity of the mouse.

Biochemical mutants of pathogens may be used to explore the biochemistry of sites of inoculation or localization in susceptible hosts. In its most simple form, a pathogen causing death rather than infection would be desirable. Death following inoculation of a mutant with a specific requirement would indicate the presence of the specific requirement. Decrease or loss of virulence, however, need not indicate absence of the requirement. The requirement may not be satisfied due to its extremely low concentration or the presence of antagonists or antimetabolites, for example.

Summary.—Five amino-acid and one pyrimidine-requiring mutants of *Klebsiella pneumoniae* were as virulent as the parental strain when inoculated into mice either intraperitoneally or intranasally. Purine-requiring mutants were avirulent when inoculated by either route. Virulence was restored with reversion to purine independence. The addition of purine to the peritoneal cavity of mice inoculated with a purine-requiring mutant inoculated by the same route resulted in death. The use of bio-

chemical mutants of a pathogen to explore the biochemistry of sites of inoculation or localization in susceptible hosts is suggested.

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THE ROLE OF OXYGEN CONCENTRATION IN DETERMINING THE EFFECTIVENESS OF X-RAYS ON THE ACTION OF A SPECIFIC GENE IN DROSPHILA MELANOGASTER

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The discovery by Thoday and Read[1] that the oxygen concentration at the time of treatment with x-rays profoundly affects the production of chromosome breaks in *Vicia faba* has resulted in a great deal of work on this and related phenomena. Geneticists have been particularly interested in the effect of anoxia on gene and chromosome mutation and the light which is thereby thrown on the nature of the mutation process.[2–12] Other workers have been equally interested in the bearing of the finding on the protection of animals from irradiation damage and in the mechanism of the protective action exerted by antioxidants.[13–15] Since it had been found by the authors that x-ray treatments of embryos or early larval stages of *Drosophila melanogaster* will block the action of a specific suppressor gene and allow the normally present but suppressed mutant gene "erupt" to manifest itself in the adult stage,[16–18] it seemed of obvious interest to test the effect of oxygen concentration upon the action of the x-rays on this developmental process.

Two genetic stocks of *D. melanogaster* were used for comparison in the study: (1) suppressor-erupt stock, *Su-er bw; st er*, which had been made isogenic and had been maintained thereafter by close inbreeding; and (2)