were available has shown that, with the exception of the northern section of the New World, the season of birth is largely meteorologically controlled.[3] This does not appear to be the case in either Puerto Rico or the northern continental United States. It would be expected that both these countries would reflect portions of the typical European pattern. Puerto Rico, at the beginning of the war, resembles a basic European pattern, which over a period of 14 years becomes analogous to that of the continental United States. On the other hand, during the last 42 years the continental United States has exhibited a relatively stable pattern in its birth season. At the same time, it is not an improbable hypothesis that the island of Puerto Rico showed a relatively stable, European-type birth pattern until after the war. The question naturally arises as to the mechanism for such a major change. Since the change occurs from a European-type pattern to an American one, it would not be presumptuous to assume that this also implies a greater contact with things American. It seems unlikely that changes of this kind could be due to large groups of itinerant travelers from the continent. It is more likely that mass communication in the form of daily newspapers, radio programs, and television shows is responsible for bringing about such a change. The most popular television programs in Puerto Rico are continental American in origin. Human interest stories and human problem-type columns are reproduced in Puerto Rican newspapers and are read avidly by the population. It is also of interest to note that many of the television programs that originate on the continent are reproduced bilingually for those Puerto Ricans who are not conversant in English. A reasonable hypothesis for bringing about such a change in such a short time among such a large group of people would be the system of mass communications.

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*THE ACTION OF STREPTOLYSIN S ON PERIPHERAL LYMPHOCYTES OF NORMAL SUBJECTS AND PATIENTS WITH ACUTE RHEUMATIC FEVER**

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The human peripheral blood lymphocyte provides a readily available *in vitro* system, representative of the donor's immune status.[1] This cell responds to specific antigens to which the donor is sensitized, as well as to certain nonspecific stimulants, such as phytohemagglutinin (PHA), an extract of the kidney bean, *Phaseolus vulgaris*. PHA has been used for the past 5 years as a mitogenic agent for lympho-

cytes, for the purpose of chromosome studies.[2] When lymphocytes are cultured for 3–5 days without any additive, the great majority remain small. After 72 hr. of culture in the presence of PHA, nearly all of the cells become large or go into mitosis. In the presence of specific antigens to which the donor of the cells has been sensitized, there is a variable increase in the percentage of large cells and mitosis.

In a study of the effect of streptococcal toxins on these cells, it was found that streptolysin O (SLO), an antigenic product of the group A β-hemolytic streptococcus, behaved like any other specific antigen, while streptolysin S (SLS) resembled PHA in its action. In view of the possibility that SLS may have an etiological relationship to rheumatic fever,[3] we studied the cells of normal individuals, patients with acute rheumatic fever, and other pertinent subjects.

Methods.—The culture methods have been previously described in detail.[4] Relatively pure human peripheral blood lymphocytes are washed and suspended in medium consisting of Eagle's minimal essential medium modified for suspension culture (MEM-S)[5] with 20 per cent fetal calf serum and 1 per cent of 200 mM L-glutamine, which has been kept frozen until used. The medium also contains 100 u penicillin and 100 μg streptomycin per ml. The final concentration of cells is 750,000 per ml. Cultures are set up in 4-ml replicates at 37°C.

PHA (Difco, type M) is added at a concentration of 0.1 ml per 4 ml of culture, while SLS and SLO are added as 50 hemolytic units per 4 ml of culture (3×10^6 cells). SLS and SLO were prepared by the method of Bernheimer.[6, 7]

Cultures were harvested (PHA at 72 hr; SLS, SLO, and control at 5 days), fixed, and stained on slides with 0.5 per cent acetic orcein. Slides were examined with phase illumination, and quantitation of response was achieved by classifying at least 1000 cells as large, small, or in mitosis. Degree of response is defined as the percentage of large cells plus mitoses in excess of the percentage found in the control cultures with no additive.

The following persons were studied: 10 patients with acute rheumatic fever not treated with penicillin (7 females, 3 males, ages 4–44), 15 normal individuals of similar age and sex distribution, 5 patients with acute rheumatic fever treated with penicillin for 2–14 days, 2 patients with inactive rheumatic heart disease, 8 patients with untreated streptococcal pharyngitis of 1–4 days duration, 1 patient with nearly fatal streptococcal septicemia treated with penicillin for 2 days, 2 patients with acute rheumatoid arthritis, 1 patient each with acute glomerulonephritis and active tuberculosis, 3 patients with agammaglobulinemia (1 congenital, 2 "acquired"), 2 infants (4 and 10 months), and 1 newborn.

Results.—The results are listed in Table 1. Control cultures without any additive generally produced the low rate of large cell formation and mitosis found in previous experiments.[1] The few exceptional cases in whom higher response was observed were later found to demonstrate penicillin sensitivity in culture. Repeat cultures without the addition of penicillin to the medium produced responses in the normal range of 5–10 per cent. PHA also produced the expected high response in the subjects studied. SLO in a concentration of 50 hemolytic units per 3 million cells produced a response as previously described for other specific antigens[1] in all individuals showing a detectable titer for anti-SLO in their serum. Cells from patients with acute rheumatic fever responded with a range of 10–47 per cent large cells plus mitoses (mean = 21.6%), while the normal group showed a response of

TABLE 1

LYMPHOCYTE RESPONSE TO PHA, SLO, AND SLS

Sex	age (yr)	Diagnosis*	Treatment	% Large Cells plus Mitoses in 1000 Cells Control	PHA†	SLO†	SLS†
F	25	N	None	5	80	28	48
F	10	N	"	5	44	24	43
M	4	N	"	26	52	4	44
F	15	N	"	11	74	26	54
M	8	N	"	5	85	20	84
F	43	N	"	8	86	18	69
M	30	N	"	3	90	14	74
F	7	N	"	10	87	26	80
F	10	N	"	4	93	22	90
F	8	N	"	9	83	10	78
F	18	N	"	7	86	30	58
M	21	N	"	11	81	15	62
F	5	N	"	5	91	25	87
M	7	N	"	9	83	8	73
F	11	N	"	4	81	11	49
M	7	ARF	ASA (2 days)	5	80	11	3
F	11	ARF	None	5	42	14	5
F	4	ARF	None	4	85	34	9
F	28	ARF	ASA (6 days)	3	81	47	14
F	10	ARF	ASA (6 days)	8	82	20	4
M	6	ARF	None	5	87	14	9
F	44	ARF	"	6	81	26	15
M	32	ARF	"	10	74	12	6
F	9	ARF	ASA (2 days)	9	82	28	8
F	15	ARF	None	12	78	10	15
F	8	ARF	ASA + penicillin (14 days)	8	72	20	55
F	11	ARF	ASA + penicillin (2 days)	34	50	16	16
M	22	ARF	ASA + penicillin (13 days)	23	62	14	62
F	9	ARF	ASA + penicillin (15 days)	9	76	10	41
F	8	ARF	ASA + penicillin (10 days)	11	83	27	58
F	47	RHD	Digitalis (4 yr)	5	80	64	76
F	24	RHD	None	5	80	71	74
M	19	SP	"	5	80	28	54
M	22	SP	"	5	64	18	8
F	20	SP	"	37	57	26	47
F	8	SP	"	12	73	25	66
F	22	SP	"	5	88	30	84
M	24	SP	"	12	79	25	68
F	7	SP	"	8	86	24	79
M	21	SP	"	3	90	28	87
M	43	SS	Penicillin (2 days)	8	52	14	10
F	39	RA	ASA (4 months)	39	38	3	41
F	42	RA	ASA (1 month)	10	86	8	77
F	14	AGN	None	5	80	27	37
F	23	TBC	INH, PAS (14 days)	6	81	12	74
M	9	CAG	Antibiotics (8 yr)	8	84	0	79
M	5	AAG	Antibiotics (4 yr)	10	81	1	72
F	31	AAG	Antibiotics (7 yr)	6	90	2	84
F	10/12	N	None	24	58	3	36
M	4/12	N	"	3	89	1	83
F	0‡	N	"	10	88	0	86

* N = normal, ARF = acute rheumatic fever, RHD = rheumatic heart disease, SP = streptococcal pharyngitis, SS = streptococcal septicemia, RA = rheumatoid arthritis, AGN = acute glomerulonephritis, TBC = tuberculosis, CAG = congenital agammaglobulinemia, AAG = "acquired" agammaglobulinemia.
† Per cent increase over control cultures with no additive.
‡ Cord blood.

4–30 per cent (mean = 18.7%). SLS in a concentration of 50 units per 3 million cells produced a range of response by cells from normal individuals of 43–87 per cent, while cells from patients with acute rheumatic fever not treated with penicillin showed a response of only 3–15 per cent. This difference is highly significant with a p value of less than 0.001 (Table 2). None of the patients were on steroid therapy. The presence or absence of salicylate therapy had no effect on the results. Re-

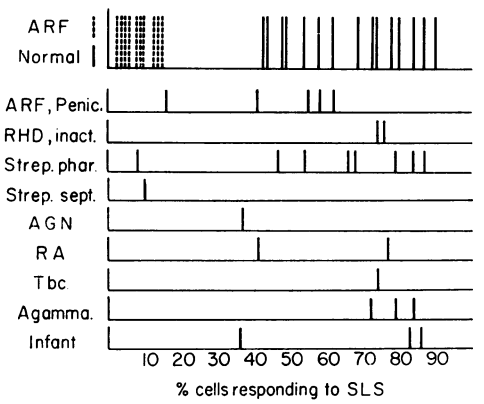

FIG. 1.—Percentage large cells and mitosis after stimulation with streptolysin S. The key to abbreviations may be found in the footnote to Table 1. All percentages are increases over large cells and mitoses found in control cultures without additive. Each experiment represents the percentage in 1000 cells.

sponses to SLS similar to those found in normals were observed in the four patients with acute rheumatic fever who had been treated with penicillin for 10–14 days, while one such patient treated for only two days demonstrated the response observed in the untreated acute rheumatics. Normal responses to SLS were also observed in seven out of eight patients with streptococcal pharyngitis, two patients with inactive rheumatic heart disease, two with rheumatoid arthritis, one with acute glomerulonephritis, and one with active tuberculosis. One patient with streptococcal pharyngitis and one with streptococcal septicemia showed the responses typical for acute rheumatics. The three patients with agammaglobulinemia and the three infants showed normal responses to SLS but did not respond to SLO. The response to SLS showed no correlation with age or sex. The responses to SLS are depicted graphically in Figure 1.

TABLE 2

RELATIONSHIP OF LYMPHOCYTE RESPONSE TO SLS AND PHA

Dx (N)	PHA	SLS	SLS/PHA
ARF (10)	77.2 ± 12.9*	8.8 ± 4.5	0.114 ± 0.054
Normal (15)	79.7 ± 13.8	66.2 ± 16.2	0.832 ± 0.133
p	>0.500	<0.001	

* Mean ± S.D.

Discussion.—When human peripheral blood lymphocytes are cultured without any additive for 3–5 days, 90–95 per cent of the cells usually remain as small lymphocytes, 5–10 per cent appear as large cells, and less than 0.5 per cent are in mitosis. After 72 hr of culture, in the presence of PHA there is an increase on the average of 75–90 per cent of large cells over the percentage found in control cultures, the rest remaining small. One to ten per cent of the cells are in mitosis. From 10 to 50 per cent of the large cells are pyroninophilic and basophilic with a perinuclear clear zone and morphologically resemble plasma cells.[1] We have previously shown that the enlarged cells contain newly synthesized gamma globulin.[8]

When the cells are cultured for 5 days in the presence of a variety of specific antigens to which the donor of the cells has been sensitized, there is an increase of

large cells, while 0.5–5 per cent of the cells are in mitosis. The antigens studied have included a variety of bacterial and viral preparations, nonprotein antigens such as penicillin, and homologous cells and cell extracts.[4]

One of the antigens we have studied is SLO in a concentration of 50 hemolytic units per million cells. All individuals showing a detectable titer of anti-SLO in their serum showed a lymphocyte response as described for other specific antigens.

In order to study the response to a nonantigenic streptococcal toxin as a control for the antigenic SLO, we simultaneously added 50 hemolytic units of SLS per million cells to replicate cultures from the same sample of blood. To our surprise, we observed that the response of the cultured lymphocytes to SLS resembled that to PHA, in that almost all of the cells enlarged or went into mitosis. Since SLS has been implicated as a possible causative agent for rheumatic fever,[3] and since Stollerman and Bernheimer[9] described the reduction of a plasma inhibitor to SLS during acute rheumatic fever, we studied a series of patients with this disease.

As seen in Figure 1, the range of response to SLS by cells from patients with rheumatic fever untreated with penicillin was significantly lower than that found in normals. Penicillin therapy for two days did not affect the diminished response, while after such therapy for 10–14 days, the response of the cells to SLS was the same as that observed in normals.

Of eight patients with streptococcal pharyngitis, only one showed the low response found in acute rheumatics. It may be of interest that this one individual has a family history of rheumatic fever. One patient with nearly fatal streptococcal septicemia, a disease in which there also may be an inadequate defense to the streptococcus, showed a response in the range of the acute rheumatics.

Three patients with agammaglobulinemia, two of the congenital variety and one of the acquired, showed normal responses to SLS, despite the fact that they did not respond to SLO. Such patients have not been found to respond to any specific antigen in culture, despite attempts at immunization *in vivo*, while their cells do show a normal response to PHA.[10] Also, in order to test whether previous exposure or immunological response to the streptococcus has any bearing on the *in vitro* response to SLS, we tested three infants: one newborn, one 4-month-old, and one 10-month-old. These three also showed a normal response to SLS. Their response to SLO was negative.

This would indicate that SLS is a nonspecific stimulant to lymphocytes, similar to PHA, but that the responsiveness of these cells is diminished during acute rheumatic fever. In order to test whether this diminished responsiveness is generalized or specific for SLS, we simultaneously tested the same individuals' cells with PHA. The cells of acute rheumatics showed a reaction to PHA no different from that of normals, while their reaction to SLS was significantly diminished (Table 2). In the last column of Table 2 it may be seen that normal individuals showed a response to SLS on the average 83 per cent as high as that to PHA, while acute rheumatics showed SLS response of only $11\frac{1}{2}$ per cent on the average of that found with PHA.

When the cells from the acute rheumatics were stimulated with SLO, they showed a range of response similar to that expected after incubation with specific antigens, indicating that the diminished response to SLS does not represent some form of immunologic paralysis. In fact, the response to SLO is somewhat higher than in

the normals, as would be expected in patients with high anti-SLO levels in their serum.

The mechanism of the normal *in vitro* response of lymphocytes to SLS is not known, but it is possible that it represents the counterpart of the *in vivo* neutralization of this substance. The diminution of this response in patients with acute rheumatic fever may then represent a loss of the ability to neutralize SLS adequately during a β-hemolytic streptococcal infection. After adequate penicillin therapy, the cells from these patients can again respond normally. In view of the high familial incidence of rheumatic fever, it is possible that a genetic defect is responsible for the loss of the postulated defense mechanism, when this is stressed by the presence of SLS in the body. This defect is expressed in the lymphocyte, but may, of course, not be restricted to this cell. Because of such a loss of neutralization, SLS may remain free in the organism and produce the tissue damage associated with acute rheumatic fever. SLS has been shown to be an agent capable of producing arthritis and damaging tissues,[11] by causing a release of lysosomal enzymes.[7] Such tissue damage may also be responsible for the apparent autoimmune phenomena described in rheumatic disease.[12] This hypothesis, that the autoimmune aspects of disease may be secondary to tissue damage and not the primary etiologic factor, has recently been proposed by Thomas.[13] We have some evidence that this may also be the reason for the presence of autoantibodies to skin in infantile eczema.[14]

If the results described are verified in future studies, the abnormally low response of cultured lymphocytes to SLS may be a more accurate clinical test for the diagnosis of acute rheumatic fever than is currently available.

Summary.—We have presented evidence that cultured peripheral blood lymphocytes show a nonspecific response to streptolysin S, similar to that found with phytohemagglutinin. This response is markedly and specifically diminished in cells from patients with acute rheumatic fever not treated with penicillin, and as might be expected, from an occasional patient with a streptococcal infection. The cells of acute rheumatics retain their ability to respond to phytohemagglutinin and specific antigens. We have presented a hypothesis that this laboratory observation may represent an inherent defect in the ability to neutralize streptolysin S when exposed to this substance *in vivo*, and that this agent then remains free to cause tissue damage.

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*RANDOM POLYGONS DETERMINED BY RANDOM LINES IN A PLANE, II**

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Introduction.—Part I of this paper, published in the previous issue of these PROCEEDINGS, presented a number of theorems and relations concerning the statistical properties of the polygons produced by random lines in a plane. The present second and concluding part discusses the methods used and an application.

Methods.—The best proofs of Theorems 1–3 are perhaps by appealing to Integral Geometry, more specifically to the facts that[5] $dG = [dp\ d\theta]$ is the line "density" that must be imposed to ensure invariance of the measure of a set of lines under coordinate transformations of the translation-plus-rotation type, and that $dG = |\sin\theta|\ [ds\ d\theta]$ is the corresponding density necessary for the lines intersecting a given curve, where such a line is specified by the length s along the curve of the point of intersection, and the angle θ made with the tangent there.

The proof of the existence of distributions, and hence of mean values, is accomplished with the aid of a multiparameter ergodic theorem of Wiener,[6] needed in the 2-parameter case here, together with a demonstration of, roughly speaking, the asymptotic independence of the realization of $\mathfrak{L}$ in regions of the plane far apart. It proves best to consider the most general case of thick lines straightaway, as then Theorem 9 occurs as a natural by-product of the theory.

Theorems 4, 5, and 8 are all special cases of a wide class of distributions[4] applicable to a homogeneous system of random r-flats (0-flat = point, 1-flat = line, 2-flat = plane, etc.) in n-space ($0 \leqslant r \leqslant n-1$: here $r = 1$ and $n = 2$) which, loosely, constitute inversions of the Poisson distribution, as exemplified here by Theorem 3. The interpretation of the two factors of the χ^2 density common to all three theorems gives a clue as to the actual method. The observation that, when every line of $\mathfrak{L}$ is given thickness w, the polygons which survive (although with reduced area) are precisely those for which $D > w$, furnishes an alternative proof of Theorem 4. For, as has been seen, the "uncovered fraction of the plane" is $e^{-\tau w}$. Application of Theorem 9 is sufficient to complete the proof.

The proof of Theorem 6 utilizes the facts that the "small" polygons of $\mathcal{P}$ are "almost all" triangles, and that there is a relatively simple random construction for a "random" triangle of $\mathcal{P}$.

Let X represent any one of N, S, A, and let $X_1, X_2, \ldots, X_{j(R)}$ be the values of X