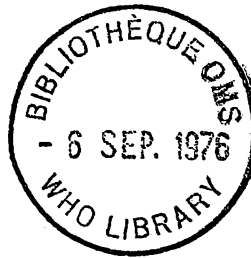




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LONGITUDINAL SEROLOGICAL STUDY OF MALARIA IN THE RURAL WEST AFRICAN SUDAN SAVANNA,
IN RELATION TO THE APPLICATION OF CONTROL MEASURES
IV. THE RELATIONSHIP BETWEEN PARASITOLOGY AND SEROLOGY

by

INDEXED

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The research project on the epidemiology and control of malaria, conducted in the Garki District, Kano State, jointly by the Government of Nigeria and WHO, included among its objectives the study of the community immune response to malaria; as detectable by multiple serological tests, before, during and after a period of intense malaria control through application of a residual insecticide and mass drug administration.

Previous papers in this series (Cornille-Brøgger et al., 1976a, b) have dealt with the study design, the methods, and the serological results. The present paper deals with the relationship between serology and parasitology in individual persons followed longitudinally in the particular epidemiological situation of the study area.

1. THE METHOD OF ANALYSIS OF THE BASELINE DATA

The longitudinal data per individual are used; they include the results of eight parasitological surveys conducted at ten-week intervals. At each of these surveys, a thick blood film was made and of the 200 fields examined the numbers found positive for P. falciparum asexual stages, P. falciparum gametocytes, P. malariae and P. ovale, respectively, were recorded. Surveys 5 and 8 were also serological surveys. At these two surveys, the population was stratified within each age-group according to the results of the six serological tests performed, i.e.: determination of serum immunoglobulins G and M; the Ouchterlony precipitin test, the indirect fluorescent antibody test (IFAT; with IgG conjugate), and the indirect haemagglutination test (IHAT) for P. falciparum; and only the IFAT (with IgG conjugate) for P. malariae. The strata were defined so that with a small number of fixed strata relatively large sub-groups would be obtained in as many age-groups as possible. The strata were compared in terms of parasitological findings before (either the immediately preceding survey, or the sum of all the preceding surveys), during and after (either the immediately following survey, or the sum of all the following surveys) the serological survey. The parasitological variables examined included the parasite rates (the proportion of persons positive for P. falciparum, P. falciparum gametocytes, P. malariae, and P. ovale respectively) and the parasite density indices (the proportion of fields of thick blood films positive for P. falciparum trophozoites, P. falciparum gametocytes, P. malariae, and P. ovale respectively).

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On the basis of six serological tests, two serological surveys, two parasitological variables (parasite rate and parasite density), two ways of grouping parasitological surveys (either the immediately neighbouring surveys, or all surveys before and/or after), 48 tables were produced and examined for correlations (positive or negative) between the serological and parasitological findings. The results selected for presentation (Figs 1-5; Table 1 gives the numbers examined) are among those which show most clearly the relationships observed; the data not presented here show the same relationships, with somewhat more irregularities. The observed P. ovale parasitaemia was very low, and it is only for the two other species that significant relationships were detected.

2. THE PRECIPITIN-P. FALCIPARUM TEST AND P. FALCIPARUM PARASITAEMIA

Fig. 1 shows a strong negative association between the test results and the P. falciparum parasite rates in age-groups 9+ years; this association is practically the same whether one considers the parasitology before, during or after the serological survey. In the <1 year age-group, the association with parasitaemia is negative before the serological survey and positive during and after the survey. The association probably changes from a positive to a negative one around age 5. Fig. 2 shows that a negative association was also observed between the test results and the P. falciparum gametocyte rates at age 1 year + before, during and after the serological survey. This negative association appears at a much earlier age than the one between precipitins and the parasite rate. Variation of the P. falciparum trophozoite density, as a function of the test result, was very similar to the one shown for the P. falciparum parasite rate. The association with density may become negative earlier in life than the association with parasite rate. The density of P. falciparum gametocytes shows a strong negative association with the test result earlier in life than is the case with trophozoite density.

3. THE IHAT-P. KNOWLESI OR P. FALCIPARUM AND P. FALCIPARUM PARASITAEMIA

For the IHAT a P. knowlesi antigen was used at the first serological survey only. Fig. 3 shows a strong positive association of the IHA-P. knowlesi titre with the P. falciparum parasite rate in the <1 year age-group and a strong negative association at ages 9 years + before, during and after the serological survey. There is a positive association between the IHA-P. knowlesi titre and the P. falciparum gametocyte rate, in the <1 year age-group and a negative association at ages 5 years + before, during and after the serological survey. A high titre is associated with decreased gametocytaemia, and this association may precede the one with decreased parasitaemia. The association of the IHA-P. knowlesi titre with P. falciparum trophozoite and gametocyte densities shows the same trend with somewhat more irregularities. The associations between the IHA-P. falciparum titre at the second serological survey and P. falciparum parasitaemia are very similar to those just described for the IHA-P. knowlesi at the first survey.

Fig. 4 shows the relationship between the IHA-P. falciparum titre at the second serological survey and P. falciparum parasitaemia and gametocytaemia at the seven preceding parasitological surveys; for both parasite forms there is a positive association with the parasitology up to the age of 5 years and a negative one thereafter.

4. THE IFAT AND HOMOLOGOUS PARASITAEMIA

The associations in this test are less obvious than above. The numbers examined were smaller and several strata were empty or nearly so. In the case of the IFAT-P. falciparum there was a positive association between the test result and concurrent P. falciparum parasitaemia up to age 5 and no association for the older ages. In the case of the IFAT-P. malariae there was a positive association between the test result and P. malariae parasitaemia in all age-groups; strong up to the age of 19 years, it was weaker thereafter. This association is very clear between the IFA-P. malariae titre at parasitological survey 8 and the P. malariae parasite rate at surveys 1-7 (Fig. 5), and also between the IFA-P. malariae titre at survey 8 and the P. malariae parasite density at surveys 1-7.

5. THE IMMUNOGLOBULIN LEVELS AND PARASITAEMIA

Fig. 6 shows the relationship between the IgM level at survey 5, and the P. falciparum parasite and gametocyte rates at survey 6, i.e. 10 weeks later. The association is negative in most age-groups, including infants. At survey 8, there was a positive association, in infants, between the IgM level and the P. falciparum parasite rate; otherwise the trend shown in Fig. 5 was also seen, although less clearly, in the remainder of the data concerning IgM.

For the IgG level, no clear cut association, either positive or negative, with parasitaemia, was found by the approach adopted.

6. THE RELATIONSHIP BETWEEN PARASITOLOGICAL AND SEROLOGICAL FINDINGS DURING THE INTERVENTION PHASE

In order to take the concurrent parasitological data into account in the analysis of the serological data, the parasitological survey results have been used to classify the protected population into two main groups, namely the "persistently negative" and the "ever positive" from the onset of the intervention phase up to the serological survey considered. Those never found positive but not examined at all surveys are included in the total but not in either of the main groups. The parasitologically positive and negative groups are defined with respect to any malaria parasite for the analysis of the IgG and IgM results, with respect to P. falciparum only for the analysis of the results of the tests specific for that parasite (Ouchterlony precipitin test, IFAT-P. falciparum, IHAT-P. falciparum), and with respect to P. malariae only for the analysis of the IFAT-P. malariae results.

There were relatively few parasitological positives in the protected population and their serology did not differ greatly from that of the others, so that practically the same results were obtained for the group of negative persons as for the total protected population. Only for the Ouchterlony-P. falciparum test did a clear picture emerge. In the protected population examined by this test, the following numbers were or had been parasitologically positive for P. falciparum at least once: 51, 125, and 223 by serological surveys 3, 4, and 5 respectively. After 20 weeks of control, positives had fewer bands than negatives at ages 5 years +; after 50 weeks of control, positives had more bands at ages 1-18 years and fewer bands at ages 19 years +; after 70 weeks of control, positives had more bands at ages 1-18 years and after that there was no difference (Fig. 7).

In the protected population examined by the IFAT-P. falciparum, the following numbers were or had been parasitologically positive for P. falciparum at least once: 15, 36, and 65 by serological surveys 3, 4, and 5 respectively. At surveys 4 and 5, positives had fairly systematically higher average titres, but most of the differences were not statistically significant.

In the protected population examined by the IHAT-P. falciparum, the following numbers were or had been parasitologically positive for P. falciparum at least once: 36, 111, and 197 by serological surveys 3, 4, and 5 respectively. At survey 3, "positives" had a significantly lower geometric mean titre in the 9-18 year age-group; at survey 4, "positives" had higher average titres in most age-groups but the differences were not significant; and at survey 5, there was no systematic or significant difference.

There was no significant or systematic difference between the parasitologically positive and the others with respect to the three remaining tests: IgG, IgM, and IFAT-P. malariae. Of those protected and examined by the latter test, only 2, 7, and 10 had been parasitologically positive for P. malariae at least once by serological surveys 3, 4, and 5 respectively.

7. DISCUSSION

At the total population level, it is likely that all six tests used here are indicators both of contact with and of partial immunity to malaria, i.e. there is more malaria and a higher level of immunity in populations showing higher test results. The present study analyses these relationships within a population with a high endemic level.

What relationship should be expected between serology and parasitology in population surveys? If the serological test is specific, then in early life the association should be positive. If the test result is associated with protection, and is persistent, then in later life the association should become negative. The higher the incidence rate, the earlier the expected transition from a positive to a negative association. If the serological status of a person is more stable than the parasitological one, then the association: (a) should be clearer if the findings of several parasitological surveys are combined; and (b) may keep the same sign over relatively long periods (e.g. the 18 months of baseline), whether the parasitology is examined before, during, or after the serological survey.

The negative associations observed are tentatively interpreted as an association between serological tests and relative protection, in terms of parasitaemia and gametocytaemia. This applies to three tests which are by order of strength of the association: precipitin (*P. falciparum* antigen), IHA (*P. knowlesi* or *P. falciparum* antigen), and IgM concentration. Not surprisingly, these tests are a better (more stable) indicator of "protection status" than a single parasitological examination. None of the six tests, however, is a perfect indicator of parasitological protection, even on a population basis; the degree of protection, associated with a given level of the test, increases with age, if we rule out, as we probably can, decreasing exposure with increasing age. This does not, per se, rule out the hypothesis that the test is measuring protecting antibody; it suggests that one or more other factors (humoral or cellular) of immunity develop more slowly than what the test measures.

An alternative explanation of the negative association may be considered. The binding of antibody by parasites might, by itself, tend to produce a negative association. However, if that were the whole explanation of the negative associations actually observed, they would exist at all ages and would probably be much more obvious with the simultaneous parasitaemia than with the past and future parasitaemia; also, the negative association with gametocytaemia would not clearly precede the one with parasitaemia. In the case of IgM (not necessarily all antibody), this alternative explanation would seem untenable quantitatively.

It has been suggested (Greenwood, 1974) that the large amounts of IgM produced by persons living in endemic malarious areas may be beneficial to the parasite. The finding, within specific age-groups, of a negative association between parasitaemia and the IgM level does not support that hypothesis.

The IFA titres were not, within specific age-groups, associated with protection. The persistently positive association between the IFA-*P. malariae* titre and *P. malariae* parasitaemia may be interpreted as follows: each episode of parasitaemia produces a relatively quick and short-lived increase in titre, so that the periods with an increased titre overlap to a large extent with the episodes of detectable parasitaemia. In the case of *P. falciparum*, episodes of parasitaemia are more frequent than with *P. malariae*, and, even if each episode of parasitaemia produces a transitory increase in titre, the more rapid succession of episodes is sufficient to blur the relationship.

The term "parasitaemia", as it is used here, obviously means parasitaemia microscopically detected by the method employed (i.e. examination of 200 fields of thick blood films); even though the method is relatively insensitive, it is sufficient, for the analysis and interpretation presented in this study, that it be well standardized.

Among the six tests used (IgG, IgM, precipitin, IFA-*P. falciparum*, IFA-*P. malariae*, IHA), the IgG test was the only one for which no definite relationship with parasitaemia was demonstrated and also the only one which showed little change under the impact of malaria control (Cornille-Brögger et al., 1976 b). In Keneba, Gambia, however, a relationship between IgG and parasitaemia was demonstrated (see below).

The changing pattern of association between *P. falciparum* parasitaemia and the results of the Ouchterlony-*P. falciparum* test under malaria control may be interpreted as follows: after 20 weeks of control, the relationship is about the same as before control, i.e. the number of precipitation bands reflects immune status; persons with fewer bands are less immune and have more parasites, possibly left over from old infections; later the average number of bands

decreases; a few persons acquire new infections, and as a consequence their number of precipitation bands becomes higher than average.

The correlation between serology and parasitology has also been studied in Keneba, Gambia (McGregor et al., 1965, 1970; McGregor & Wilson, 1971; Rowe et al., 1968). In both the Garki and Keneba areas the four serological variables increased with age, while the parasite rates and densities decreased. The relationship was also studied within specific age-groups: the data from Keneba were analysed by comparing the serological results in persons found positive and negative at the concurrent parasitological examination; the data from Garki were analysed in the same manner and also by stratifying persons by their serological results and comparing the strata with respect to the preceding, concurrent or subsequent parasitological findings; it is the latter analysis which was presented in this paper. In Keneba, a positive correlation was found between IgG and the concurrent parasitaemia at ages 0-20 years, between IgM and the concurrent parasitaemia at ages 0-2 years, while in Garki, there was no correlation between IgG and parasitaemia and a negative correlation between IgM and concurrent or subsequent (10 weeks later) parasitaemia. The difference between the two areas with respect to IgG is difficult to explain; as noted, the margin of error in measurement is about the same; the difference with respect to IgM in the young children could be explained by a higher rate of antigenic stimulation and development of parasitological immunity in Garki, but the difference in adults (no correlation in Keneba, a negative one in Garki) is puzzling; it is possible that in Keneba the situation was modified by the use of drugs to a larger extent than in Garki. With respect to the IFAT-P. falciparum, both areas show a positive association between titre and parasitaemia at ages 0-4 years, and none thereafter. With respect to gametocytes, there was no correlation with the IFAT-P. falciparum titre, in Keneba, at ages 0-4 years. In the same age-group, in Garki, there was also no correlation between gametocytaemia and the IFAT-P. falciparum titre, but there was a negative correlation between gametocytaemia and the number of bands in the precipitin-P. falciparum test.

8. SUMMARY

The relationship between parasitological and serological results in the same person was studied for the specific age-groups in a situation of highly endemic malaria in the Sudan savanna. No relationship was found between IgG and parasitology. For the five other serological tests (IgM level, precipitin-P. falciparum test, IFAT-P. falciparum, IFAT-P. malariae and IHAT-P. knowlesi or P. falciparum) there was a positive association in early life between the test result and parasitaemia (with P. malariae for the IFAT-P. malariae and with P. falciparum for the four other tests). For three tests (IgM level, precipitin-P. falciparum test, and IHAT-P. knowlesi or P. falciparum) the relationship becomes negative in older children and adults, i.e. the test results are associated with protection. For the IFAT-P. malariae, the association with parasitaemia remains positive throughout life; for the IFAT-P. falciparum, no association was found after the age of five years. During the period of fairly effective malaria control by a residual insecticide and mass drug administration, the association between precipitins and parasitaemia, which was initially negative, became progressively positive, while the average level of precipitins progressively decreased.

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RESUME

La relation entre sérologie et parasitologie du paludisme chez la même personne a été étudiée longitudinalement, pour chaque groupe d'âge, dans des villages à endémicité élevée de la savane soudanienne. Il n'y a pas de relation entre IgG et parasitologie. Pour les cinq autres tests sérologiques (IgM, précipitation et immunofluorescence à Plasmodium falciparum; hémagglutination à P. knowlesi ou P. falciparum; immunofluorescence à P. malariae), il y a, dans la première ou les premières années de la vie, une association positive entre le résultat du test et la parasitémie (à P. falciparum pour les quatre premiers, à P. malariae pour le dernier). Pour trois de ces tests (IgM; précipitation à P. falciparum; hémagglutination à P. knowlesi ou P. falciparum), l'association devient ensuite négative (vers 10 ans ou plus tôt, et plus tôt pour les gamétocytes que pour les formes asexuées); en d'autres termes, les tests sont, dans certains groupes d'âge, des indicateurs de l'immunité parasitaire. Pour l'immunofluorescence, l'association avec la parasitologie reste positive dans tous les groupes d'âge pour P. malariae, alors qu'après cinq ans il n'y a plus d'association pour P. falciparum. Les associations décrites ne varient guère, que l'on considère la parasitologie avant, pendant ou après une enquête sérologique.

Au cours de la période de contrôle du paludisme - contrôle assez efficace par un insecticide et la chimiothérapie de masse - l'association entre précipitines et parasitologie, initialement négative, devient progressivement positive, tandis que le niveau moyen du résultat sérologique décroît progressivement.

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TABLE 1. DISTRIBUTION, BY TEST RESULT AND AGE, OF PERSONS INCLUDED IN THE STUDY OF THE RELATIONSHIP BETWEEN SEROLOGY AND PARASITOLOGY

Survey No.	Test	Result	< 1	1-4	5-8	9-18	19-28	29-43	44+
5	Precipitin- <u>P. falciparum</u> test No. of bands	0	13	16	6	4	4	0	2
		1	42	76	71	39	18	27	14
		2	13	109	166	117	71	130	51
		3	3	65	120	163	171	286	156
		4+	0	7	36	73	133	318	236
5	<u>IHAT-P. knowlesi</u> titre	<16	4	21	70	43	28	51	26
		16-32	7	65	110	128	92	136	90
		64-128	8	36	57	64	80	151	66
		256+	5	25	41	66	108	247	154
8	<u>IHAT-P. falciparum</u> titre	<16	11	19	19	14	6	10	3
		16-128	12	65	78	74	41	49	29
		256-1 024	9	84	159	200	173	362	199
		2 048+	2	41	53	81	118	207	136
8	<u>IFAT-P. malariae</u> titre	<20	23	9	0	0	0	0	0
		20-180	48	58	16	2	1	0	0
		540	9	29	17	17	10	7	3
		1 620	3	56	81	54	21	29	20
5	IGM, % IR 0172 Standard	4 860+	0	46	72	50	21	41	28
		1 = 1-19%	18	86	67	22	22	54	27
		2 = 20-39%	23	110	209	168	120	211	133
		3 = 40-69%	3	17	81	134	146	222	109
	4 = 70+%	2	3	28	39	98	243	164	

FIG. 1. THE P. FALCIPARUM PARASITE RATE AT PARASITOLOGICAL SURVEYS 1-4, 5 AND 6-8, ACCORDING TO AGE AND TO THE NUMBER OF BANDS (0 TO 4+) IN THE OUCHTERLONY PRECIPITIN-P. FALCIPARUM TEST AT SURVEY 5

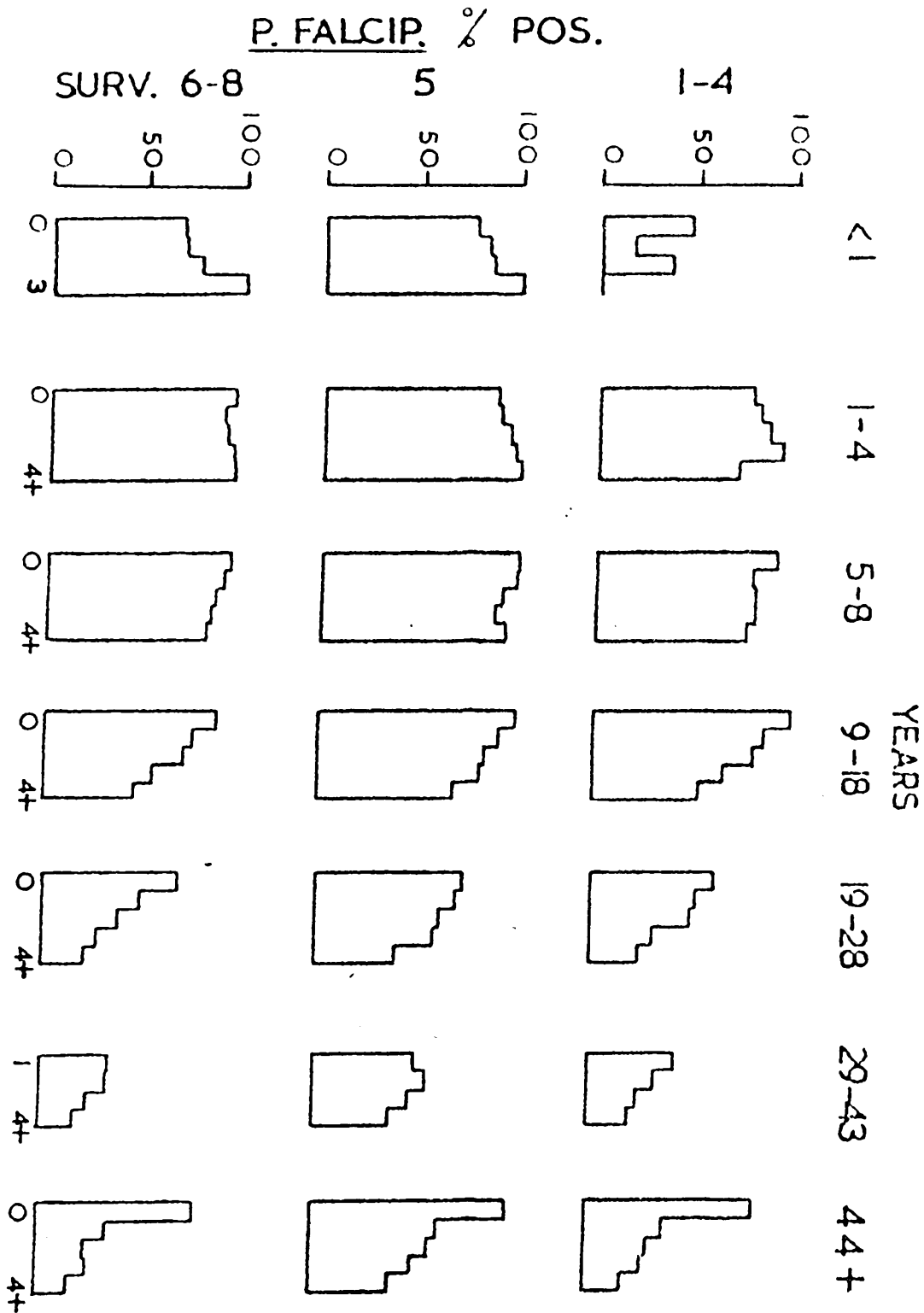


FIG. 2. THE P. FALCIPARUM GAMETOCYTE RATE AT PARASITOLOGICAL SURVEYS 1-4, 5 AND 6-8, ACCORDING TO AGE AND TO THE NUMBER OF BANDS (0 TO 4+) IN THE OUCHTERLONY PRECIPITIN-P. FALCIPARUM TEST AT SURVEY 5

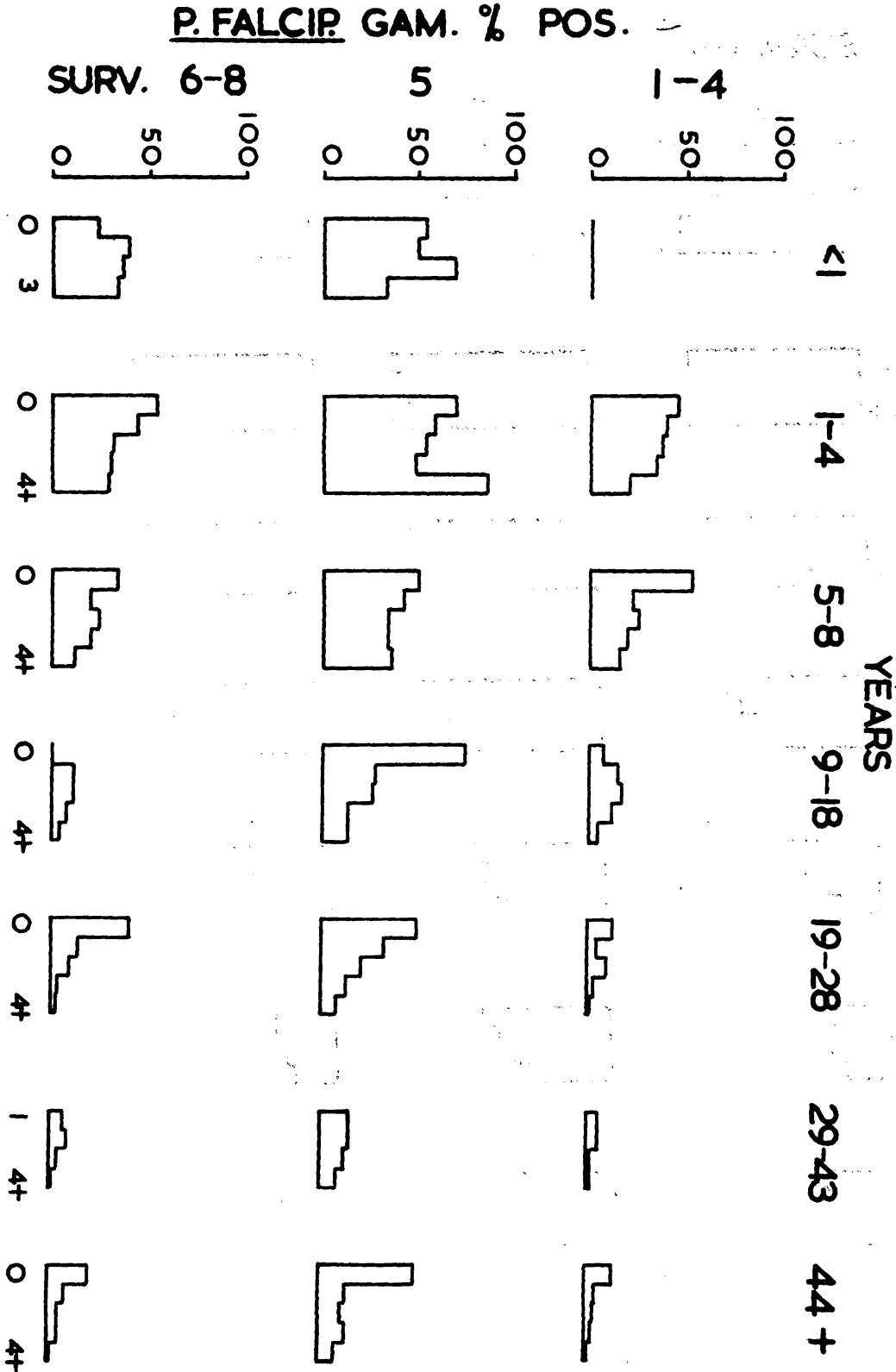


FIG. 3. THE P. FALCIPARUM PARASITE RATE AT PARASITOLOGICAL SURVEYS 1-4, 5 AND 6-8, ACCORDING TO AGE AND TO THE TITRE (<16, 16-32, 64-128, 256+) OF THE IHAT-P. KNOWLESI AT SURVEY 5

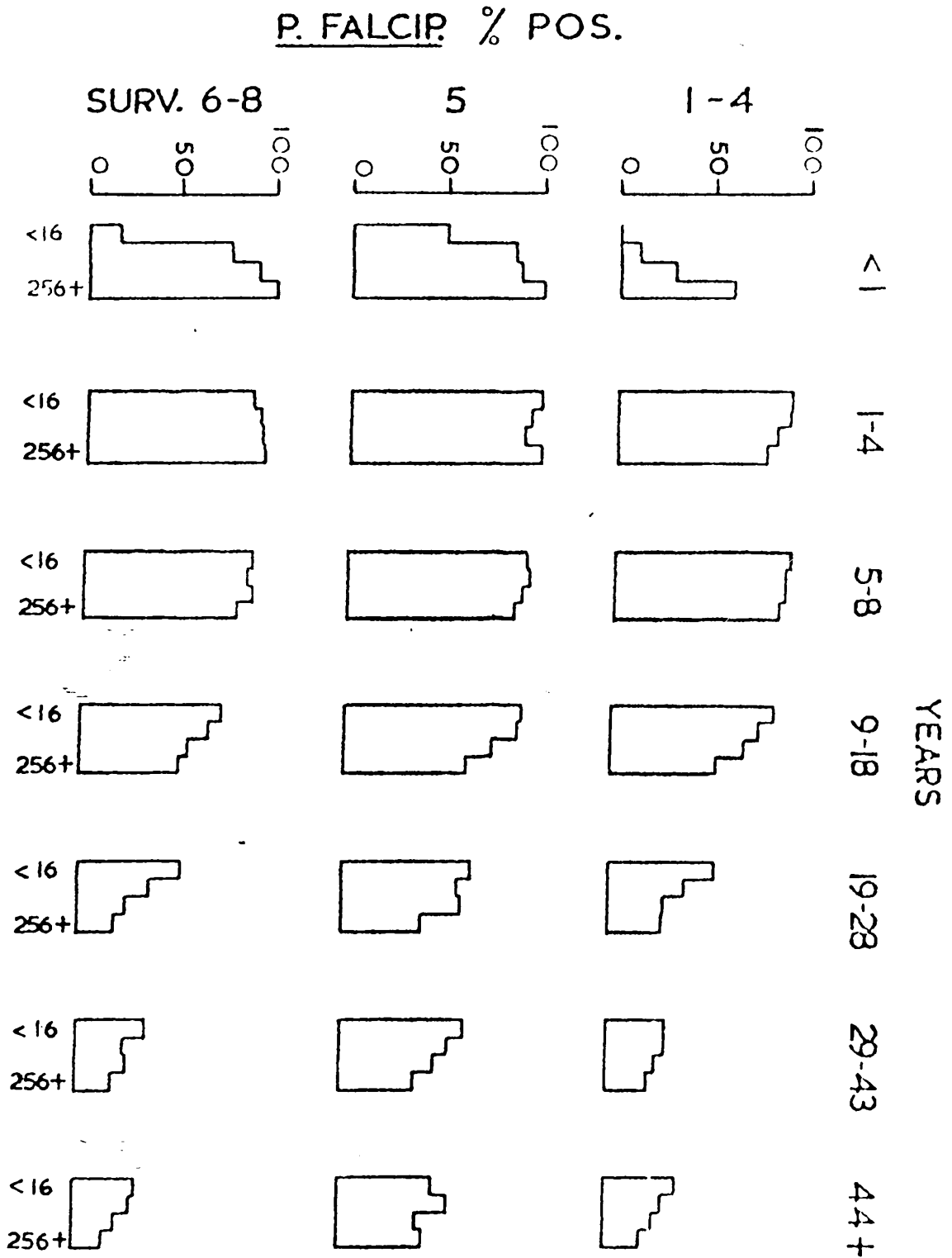


FIG. 4. THE P. FALCIPARUM PARASITE AND GAMETOCYTE RATES AT PARASITOLOGICAL SURVEYS 1-7, ACCORDING TO AGE AND THE TITRE (<16, 16-128, 256-1024, 2048+) OF THE IHAT-P. FALCIPARUM AT SURVEY 8

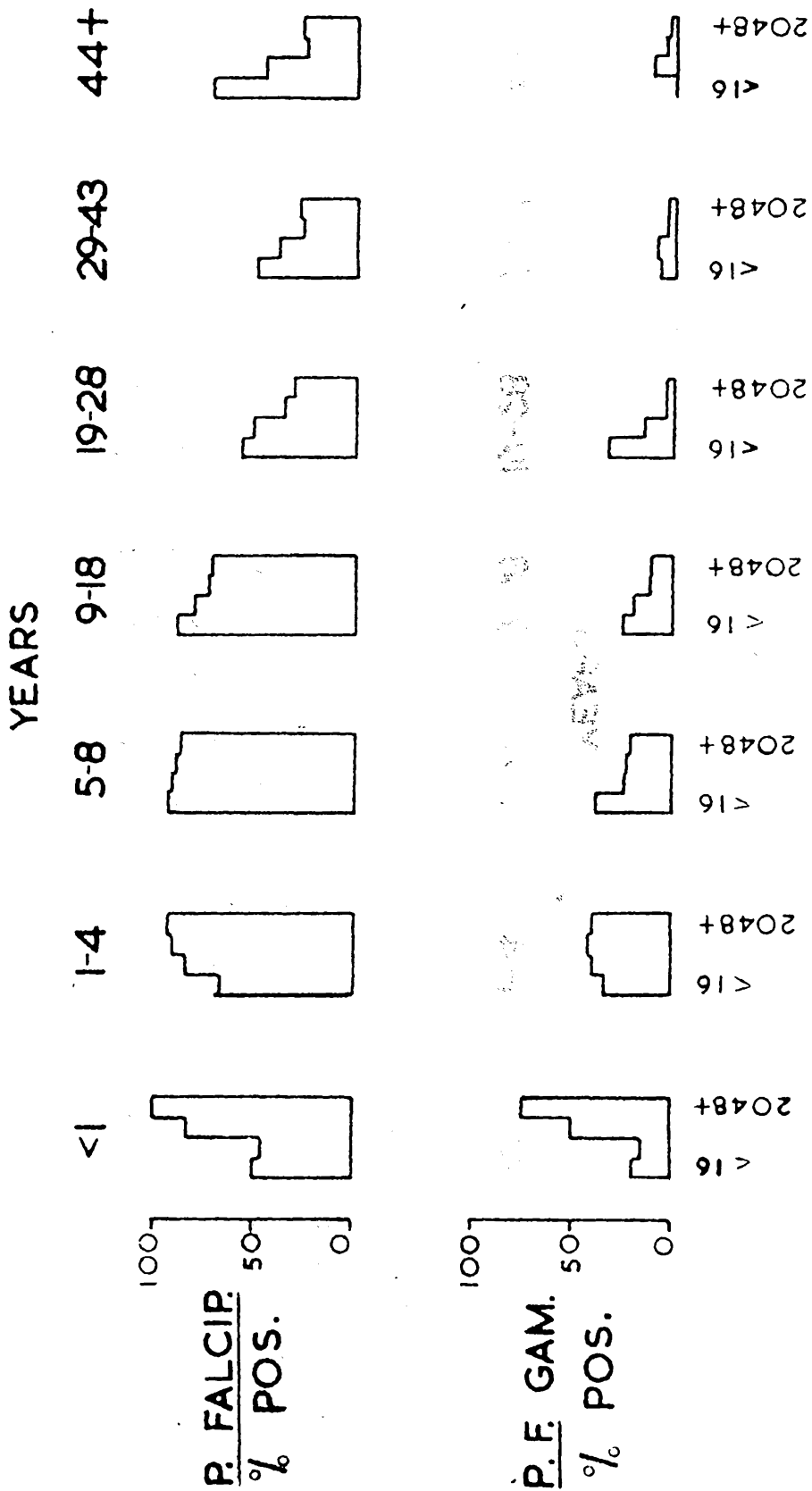


FIG. 5. THE *P. MALARIAE* PARASITE RATE AT PARASITOLOGICAL SURVEYS 1-7, ACCORDING TO AGE AND TO THE TITRE (<20, 20-180, 540, 1620, 4860+) OF THE IFAT-*P. MALARIAE* AT SURVEY 8



FIG. 6. THE P. FALCIPARUM PARASITE AND GAMETOCYTE RATES AT PARASITOLOGICAL SURVEY 6, ACCORDING TO AGE AND TO THE LEVEL OF I_gM AT SURVEY 5 (1, 2, 3, 4, = 1-19, 20-39, 40-69, 70+% OF THE IR 0172 STANDARD)

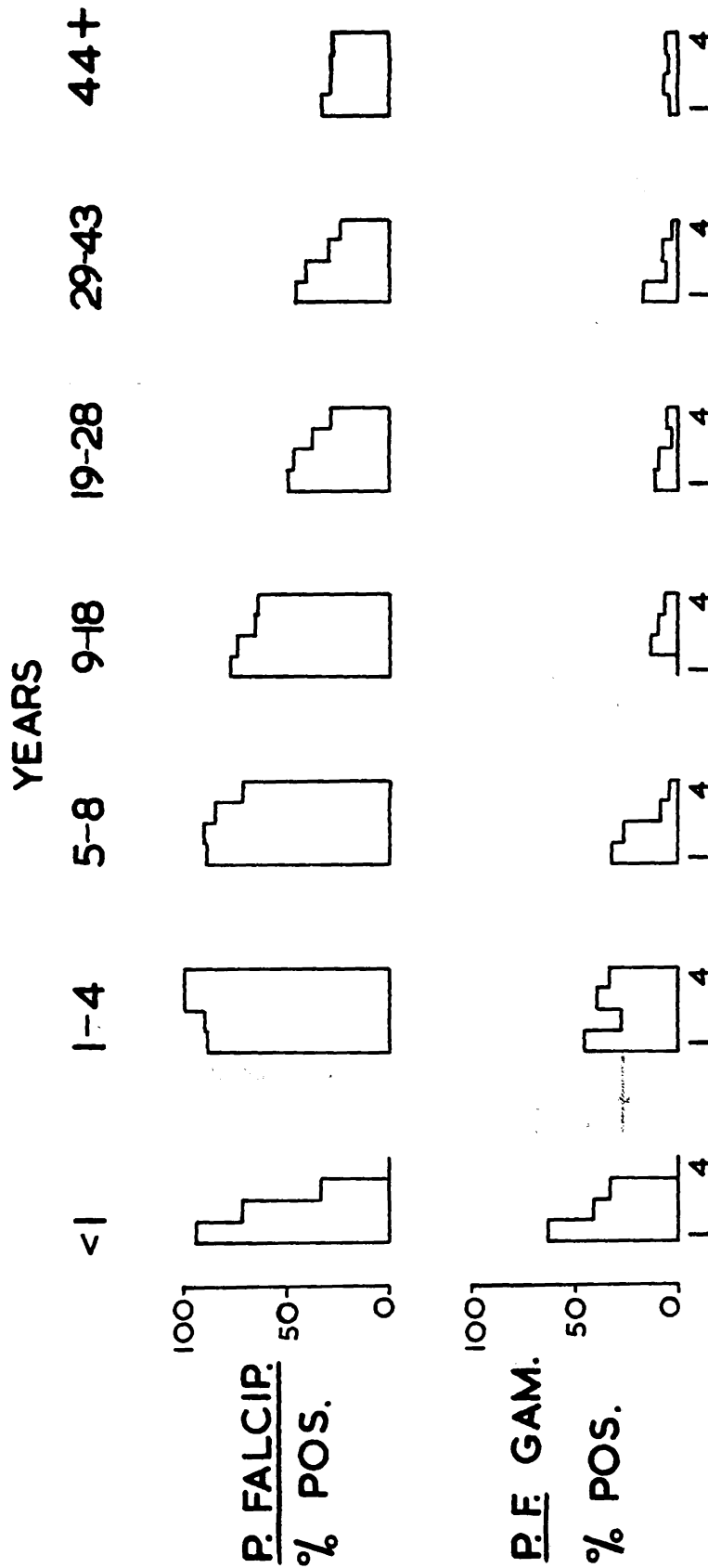
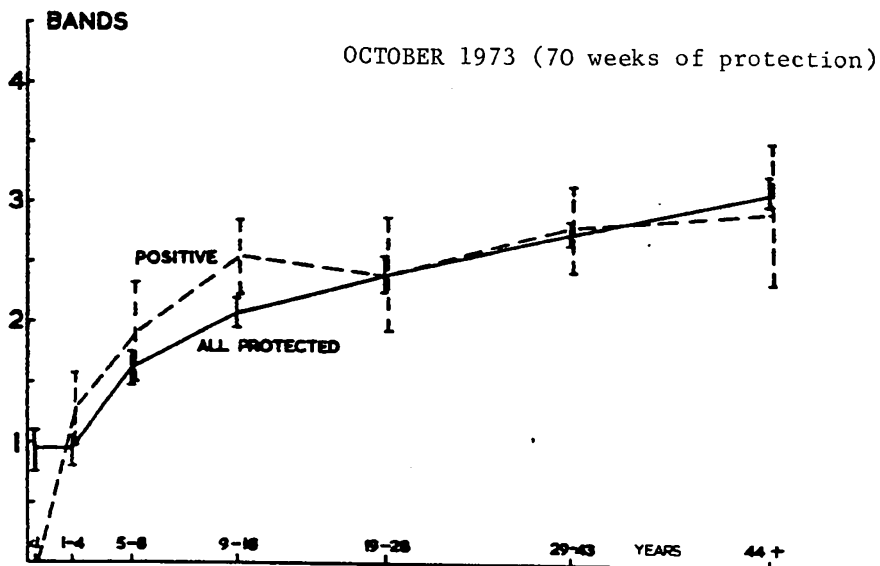
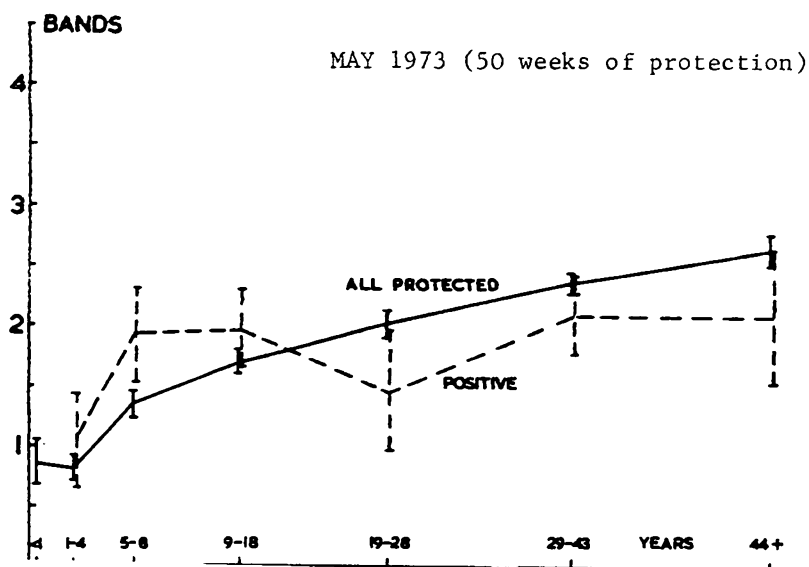
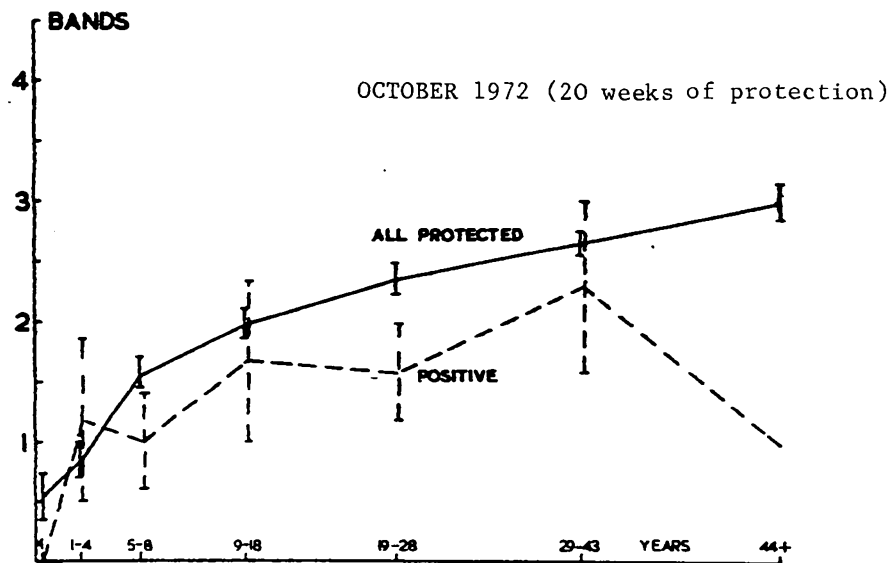


FIG. 7. THE NUMBER OF PRECIPITATION BANDS IN POSITIVE PERSONS VERSUS ALL PROTECTED PERSONS (NEARLY ALL NEGATIVE), AFTER 20, 50 AND 70 WEEKS OF PROTECTION



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