with anti-A did not affect their reaction with anti-B, and vice versa, so that the changes in the A and B antigens appear to be quite independent of one another, and it is extremely difficult to imagine any enzyme—or indeed any other agent—which can affect AB cells in this way.

Morgan and Watkins (1956) showed that the group-specific substance in the secretions of group AB people is not a mixture of separate A- and B-substances but a single AB substance. Although it is not known whether this applies to the lipoidal group-specific substance of the red cells, this might well be so, and would make it even more difficult to imagine any agent which could change some AB cells to A and others to B.

Thus the results of the study of transfused cells and of the patient's own cells were complementary and led to the conclusion of chromosomal changes in the red-cell precursors, and it was of interest that a large number of stem cells were present in the patient's bone-marrow and peripheral blood. If chromosomal changes occurred in the stem cells one could imagine that some would develop into erythrocytes with defective antigens and others into leukaemic leucocytes.

In the case described by Gold *et al.* (1959) the proportion of cells which were inagglutinable by anti-A diminished during a remission but increased again during a relapse. This change, however, need not be regarded as contrary to the idea of chromosome abnormality, since the remission was the result of treatment with 6-mercaptopurine and prednisone, and it might be supposed that this had interfered with the proliferation of abnormal stem cells to a greater extent than with that of normal ones. It is, however, less easy to understand why the red cells in the case described by Hoogstraten *et al.* returned to normal A₁ in the terminal six weeks of the illness.

Our patient's blood contained 20% O, 12% A, 42% B, and 26% AB cells, and this means that 62% of his cells had lost their A antigen and 32% their B antigen, and it is interesting that the proportion of O cells present (20%) was equal to 62% × 32%. This, of course, may be fortuitous, though 6,600 cells were counted in these experiments and it was thought that the percentage of O cells found was unlikely to be more than 3% in error. If it is not fortuitous it supports the idea that the O cells are the end-result of two independent processes, and it also suggests that the precursors of all the patient's erythrocytes had been exposed to damage, since the presence of normal AB cells which had not been involved in the leukaemic process would have given a different set of percentages.

The idea that all the erythrocytes present might be of abnormal origin is supported by a consideration of the A₁-A₂ subtypes. The patient's genotype from the family study was A₁B, yet no A₁B or A₁ cells were found in his blood, though tests on mixtures of A₁B and A₂B cells showed that as few as 2% of A₁B or A₁ cells would have been easily detected if they had been present, and there was no difficulty in detecting A₁ cells in his circulation after transfusion with A₁ blood. The fact that transfused A₁ cells reacted normally with anti-A₁ after 12 days in the patient's circulation suggests that the loss of the A₁ antigen took place at the chromosome level.

It is of interest that in many of the cases of this phenomenon which have been recorded only the ABO groups have been affected, though changes in the antigens of some of the other blood-group systems have

been described, and it would seem that changes in the A and B antigens would, for technical reasons, be easier to detect than changes in the other antigens.

Summary

The red cells of a patient suffering from eosinophilic leukaemia, and known from family studies to be of genotype A₁B, were only partially agglutinated by anti-A and anti-B. The abnormality of the A antigen was independent of the abnormality of the B antigen, and the patient's blood was shown to contain A₂B, A₂, B, and O cells but no A₁B or A₁ cells. Similar changes could not be induced in normal red cells *in vitro*, nor in the patient's circulation.

These findings can only be explained by supposing that changes had taken place in the chromosomes concerned with the ABO groups.

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Preliminary Communications

Dermal Leishmaniasis in British Honduras : Transmission of *L. brasiliensis** by *Phlebotomus* Species

Phlebotomus species have long been the primary suspects concerned in the transmission of leishmaniasis wherever the disease occurs throughout the Old and the New World. The geographical distribution of this insect coincides with that of leishmaniae, and infected *Phlebotomus* have been found in nature. Furthermore, leishmaniae have been experimentally transmitted to volunteers by these insects (Adler and Theodor, 1957, review). Nevertheless, experimental transmission has always remained difficult to achieve, and we feel that the present observations on the transmission of *Leishmania brasiliensis* by *Phlebotomus* in British Honduras are worthy of note. Clinical and other aspects of cutaneous leishmaniasis in this region of Central America have already been discussed by Biagi F. (1953) in Mexico and by Garnham and Lewis (1959) in British Honduras.

**Leishmania tropica mexicana* is the taxonomy preferred by some authors.

THE PARASITE

The parasite strains used throughout this work were isolated in N.N.N. medium nine months previously from human lesions. They were immediately transferred to laboratory animals, by which means we hoped to avoid any possible attenuation of the strains and consequent loss of infectivity due to repeated passage in N.N.N. medium.

CHOICE OF EXPERIMENTAL ANIMAL

As indicated by Garnham and Lewis (1959) the hamster (*Mesocricetus auratus*) is found to develop large dermal lesions readily at the site of intradermal inoculation of leptomonads: these lesions contain vast numbers of leishmaniae. We find that white mice are almost as readily infected, but their small size is a disadvantage in transmission experiments. Very poor infections were obtained in white rats, guinea-pigs, and gerbils.

THE VECTOR: PHLEBOTOMUS SPECIES

Although we are able to breed *Phlebotomus* species in the laboratory we are unable to do so in numbers sufficient for experimental purposes and have as a result resorted to using wild-caught females. This method has the advantage of supplying an inexhaustible number of man-biting females, every one of which (provided they are collected off human bait) is physiologically conditioned to feed immediately. The possibility of naturally infected flies being present in our catches does not appreciably influence the present findings, for this would in itself be evidence that *Phlebotomus* is a natural vector. At the most, such flies might obscure data on the time taken for the parasite to develop fully in the experimental insect—that is, the time taken for the parasite to reach the biting mouth-parts. The extremely small percentage of naturally infected *Phlebotomus* in our catches (see below) suggests that this factor may be virtually ignored. Furthermore, all 15 females dissected five days after being infected have shown massive foregut infections, which supports the evidence submitted below that transmission may be completed within four to five days.

Transmission Experiments

A series of pilot experiments led to the adoption of the procedures outlined below, which produced a 100% infection rate.

A temporary laboratory is established near to the collecting-site yet far enough away to prevent flies from being attracted from the trapping area by light. The nocturnal (see Table) females are caught off human bait

Phlebotomus Females Caught Off and by the Same Two Human Baits Throughout a 24-Hour Period. This Illustrates the More Typical Nocturnal Feedings Habits of the Insect

Time:	P.M.			A.M.		
	12-4	4-8	8-12	12-4	4-8	8-12
<i>Phlebotomus</i> ..	17	89	425	439	73	0

—each fly in a 3 by 1 in. (7.5 by 2.5 cm.) glass specimen tube containing a green leaf. The tubes containing the flies are conveyed immediately to the temporary laboratory, where they are placed on the shaved lesions of manually restrained hamsters. The females usually feed immediately, and if not they are rejected. Next morning the fed flies are transported to the main laboratories and the tubes are placed on moist cotton-

wool in a glass humidity chamber which is exposed to indirect sunlight. The latter is necessary for photo-synthetic action of the leaves, whose function it is to provide oxygen, remove carbon dioxide, help maintain a high humidity, and provide an alternative resting surface to glass. A weak sugar solution is applied daily to a strip of filter paper which projects into the tube and is held in position by the cork. Grooves cut in the cork allow air exchange.

A total of 100 females were infected as described above, and all subsequent feeds were on human volunteers on whom the bites were mapped accurately. Several females which at first refused to feed were induced to do so by rapidly cooling the tube containing them. The following *Phlebotomus* species either re-fed completely or probed in the attempt to re-feed: 13 flies (7 *P. ylephiletor* Fairchild and Hertig, 5 *P. geniculatus* Mangabeira, and 1 *P. paraensis* Costa Lima) for a second time; five (all *P. ylephiletor*) for a third time; and one (*P. ylephiletor*) for a fourth time. On dissection one day later, the latter's mouth parts were found to contain mobile leptomonads, which, although the volunteer was not infected, supports the accepted theory that transmission is by bite.

A small "pimple" (3 mm. in diameter) developed at the site of one bite on the mid-abdominal region of one volunteer 17 days after the insect had probed. Seven days later a small incision was made into the base of the swelling and exudate was used for N.N.N. culture and preparation of stained smears. Both proved positive. Twenty-eight days after the infective bite the lesion was surgically removed, fixed in Carnoy's solution, and stored in 70% alcohol for subsequent sectioning.

The vector was identified as *P. paraensis* Costa Lima. Since it is the generally accepted view that transmission occurs at the third blood meal, it is of considerable interest that in this case transmission was achieved at the second blood meal—only four days (less nine minutes) after the fly had been infected. Also of interest is the fact that the infective bite of this fly was merely a 30-second probe resulting in no blood ingestion. Possibly transmission is facilitated when a fly is disturbed before actually ingesting blood. Any parasites ejected into the wound from the mouth parts (possibly with saliva or anticoagulant) are not then sucked back with the blood meal.

Natural Infections

The demonstration of naturally infected flies forms an essential step in the incrimination of *Phlebotomus* as a vector of leishmaniasis.

Approximately 270 wild female *Phlebotomus* were trapped, using human bait. They were triturated in 2 ml. of sterile Locke's solution and one-half of the suspension was inoculated intradermally into the back of a clean hamster.

Two months later a small dermal swelling was noted at the site of inoculation. Stained smears from the incised lesions revealed leishmaniae and culture in N.N.N. produced leptomonads.

The dissection of newly caught man-biting *Phlebotomus* has revealed leptomonad-like flagellates in only 2 flies out of the 334 examined. In the first case (*P. ovallesi* Ortiz) a small cluster of flagellates was located in the hind-gut, immediately behind the malpighian tubules. In the second instance (*P. cruciatus* Coquillett) the presence of scanty flagellates was detected only after crushing the gut, and the location in the insect

was uncertain. In view of the scanty infections and the position in the hind-gut in one instance, we think that they are more likely to represent species of *Leptomonas* or *Herpetomonas* rather than leptomonad stages of *Leishmania*.

A more detailed account of these and further studies will be published in the near future.

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Medical Memoranda

South American Leishmaniasis in London

Mucocutaneous or South American leishmaniasis is rarely seen in this country but is endemic throughout Latin America, with a particularly high incidence in certain parts of Peru and Brazil. The disease is also known to occur in the Guianas. Snow *et al.* (1948) report some nine members of the American Forces and three labourers who became infected when serving in the Panama region during the 1939-45 war.

An early case of South American mucocutaneous leishmaniasis which occurred in a negro from British Guiana and presented in London is reported below.

The causative parasite is *Leishmania brasiliensis*, which is transmitted by the bite of several species of sandfly (*Phlebotomus*). The parasite proliferates in the gut of the sandfly, while man, dogs, wild rodents, and possibly other species serve as reservoir hosts. Unlike kala-azar and Oriental sore, mucocutaneous leishmaniasis is found most commonly among rural communities in warm wooded areas with high rainfall. The higher incidence in males is thought to be purely occupational.

The incubation period is usually rather short, being about two to four weeks. The initial lesions are papules which develop at the site of sandfly bites and may be single or multiple, and occur on the exposed surfaces of the skin. The primary lesion may regress spontaneously (abortive course), remain stationary, develop into nodules or vegetative lesions, or, as is more usual, undergo ulceration. Chronic ulcerating and vegetating lesions of the nasobuccopharyngeal mucosa are a characteristic feature of the disease and are probably the result of lymphatic or blood-stream dissemination, although local extension from the skin may occur. The frequency of mucous membrane lesions varies from region to region. The lymphatics draining the primary lesion may become thickened and develop nodules along their course. These nodules occasionally break down in the centre, forming fluctuant masses which may eventually ulcerate (Snow *et al.*, 1948). This lymphangitic form bears a striking resemblance to sporotrichosis. In spite of the extensive nature of the skin lesions the patients usually feel well and have minimal lymphadenopathy without visceral involvement.

This diagnosis is confirmed by demonstrating Leishman-Donovan bodies in smears or sections taken from the non-ulcerated skin at the edge of a lesion. Some of the material so obtained may be sown on to N.N.N. medium (saline agar plus defibrinated rabbit blood), be incubated at 22° C., and the flagellate forms of the parasite demonstrated.

CASE REPORT

A 27-year-old male negro diamond-diver from British Guiana first attended hospital in April, 1961, five days after arriving in England. On board ship, two weeks before landing in England, he noticed a "boil" on the forehead, and during the next 10 days many more similar lesions occurred on the face, arms, and legs.

When he presented at the out-patient department two types of lesion were present. On the face (Fig. 1) there were multiple, secondarily infected granulomatous lesions up to 1 cm. in diameter, while on the arms and legs (Figs. 1 and 2) there were larger, deeper nodules, some of which were fluctuant and measured up to 4 cm. in diameter. Within the nasal cavity the lateral walls, septum, and upper surface of the soft palate showed a fine nodularity and the blood-vessels were dilated. No lesions were seen in the pharynx or larynx. Apart from minimal lymphadenopathy and a low-grade pyrexia of 99° F. (37.2° C.) no other abnormality was found. However, one week after admission to hospital and before any treatment had been instituted he developed a painful red swelling of the interphalangeal joint of his left second finger.

Investigations.—Haemoglobin, white blood count, and differential white blood count were all normal. E.S.R. (Wintrobe) 32 mm. in one hour. Total serum proteins

FIG. 1