IDENTIFICATION OF BLOOD MEALS OF BLOOD-SUCKING ARTHROPODS

B. WEITZ

Lister Institute of Preventive Medicine, Elstree, England

SYNOPSIS

The identification of blood meals of blood-sucking arthropods requires a test which is sensitive enough to detect even partially digested blood and specific enough to identify the various hosts. The technique of preference is the precipitin test which makes use of the specific combination of the serum proteins of the blood. The preparation of the blood meal extract and the procedure for the absorption of the antisera is described and various methods of performing the precipitin test are mentioned. When the hosts involved are closely related to each other it is necessary to use the inhibition test. The blood meal will specifically inhibit, under specified conditions, the agglutination of tanned and sensitized red blood cells which is caused by a suitable antiserum. The procedure adopted for the identification of blood meals derived from a large variety of hosts is described in detail.

The study of the bionomics of insect vectors of disease requires accurate information of their feeding habits and preferences. Different techniques have been employed by various workers for this purpose, but, it must be admitted, in many instances without sufficient attention to the details of the techniques used, with the result that the interpretation of the work is at times left in doubt.

The precipitin test has been used most commonly, and as it is undoubtedly the most useful test for the identification of blood meals it is described here in relation with other methods which also have been employed.

Whichever method is selected, the test of choice is one which is, at the same time, highly sensitive and specific.

SENSITIVITY

A high degree of sensitivity is required because, in many cases, the insect meals may only contain a very small proportion of identifiable protein. The feeding capacity of the insect involved is one of the most important limiting factors in this respect. Arthropods, such as the tick, particularly the larger ones, e.g., *Ornithodorus* species, take a comparatively large

volume of blood, while some of the serum sucking mites, e.g., *Bdellonyssus bacoti*, will take up only a very small meal. Between these two species of blood-sucking arthropods there is a very wide range of size of blood meals taken up by different insects. Apart from the content of the blood-meal at the time of feeding, the rate of digestion of the identifiable protein varies considerably between insects. Weitz & Buxton [14] found that the antigenicity of the protein in the stomach contents of *Ornithodorus moubata* fed on fowl was preserved more than 210 days after feeding, as recognized by the precipitin test, and there is a report by Gozony, Hindle & Ross [4] of a positive reaction obtained from a blood meal of *Argus persicus* and *Ornithodorus moubata* with fowl antiserum two years after feeding. This is a remarkable phenomenon which deserves further investigation but, it must be admitted, is quite exceptional. Most species of blood suckers have completed the digestion of their serum protein in a matter of a few days or less. Table I shows the results of tests on blood meals on a number of species at various intervals of feeding and indicates that the time during which the feeds are reliably identified by the precipitin test is extremely limited.

The habits of the insects themselves very often limit the time after feeding during which they can be captured. Domestic insects, such as many of the malarial mosquitos, can be caught quite easily in bedrooms after a feed on the inhabitants, but then it is hardly necessary to identify the blood meals, unless of course the mosquitos have taken refuge in the house after a meal outdoors. The difficulty of capturing well fed insects arises particularly among those species which, having fed either indoors or outdoors, subsequently rest outdoors. Unless their resting places are well known, a very large proportion of captured insects of such habits will inevitably contain either largely digested material in their stomachs or will be found to be empty. Bait catching is not usually a very successful method since it only attracts hungry individuals which have consequently empty stomachs. Light traps and such devices are usually more successful although in practice only a small proportion of individuals contains sufficient blood to warrant testing (Hurlbut & Weitz [6]). The environmental conditions may have a considerable effect on the longevity of the blood meal. This has been shown under laboratory conditions (West & Eligh [16]), but one of the most interesting observations relates to the marked differences of metabolic rate, and consequently to the digestion of blood meals, which are caused by captivity after feeding compared to the natural behaviour of certain insects, which are released in their natural environment after feeding. This phenomenon was noted in tsetse flies. When *Glossina morsitans* was fed and kept under captivity, the stomach contents of 95%-100% of the flies was still identifiable after 3 days, but when the marked flies were liberated in the bush and subsequently recaptured, only 5%-10% of stomach contents could be identified after approximately 3 days, whereas 80%-100% of

TABLE I. POSITIVE RESULTS OF PRECIPITIN TESTS WITH BLOOD MEALS OF ARTHROPODS AFTER VARYING PERIODS OF DIGESTION

Species of arthropod	Host	Number of positive meals per group tested after:										
		3 hrs.	6 hrs.	15 hrs.	20 hrs.	24 hrs.	30 hrs.	40 hrs.	2 days	3 days	4 days	5 days
Culex molestus	man					11/11				0/5		0/5
Culicoides nubeculosus	man					4/5				0/5		
Anopheles aquasalis [a]	ox			45/48	13/50		2/50	0/48		0/48		
Anopheles maculipennis atroparvus	man			10/10						1/11	0/10	0/2
Aëdes aegypti	man			10/10						3/11	0/10	0/19
Glossina morsitans	man	20/20				20/20			20/20	19/19		
	ox	20/20				20/20			20/20	20/20		
	sheep	20/20				20/20			20/20	15/20		
	goat	20/20				20/20			20/20	18/20		
Glossina swynnertoni [b]	mammal				I c 2/2	II c 5/9				III c 7/25	IV c 1/44	
Bdellonyssus bacoti	man	5/5				9/10				0/3		0/10

		Number of positive meals per group tested after:												
		1 day	5 days	10 days	20 days	30 days	35 days	50 days	65 days	90 days	120 days	150 days	180 days	210 days
Cimex lectularius	man	5/5	10/10	29/30	4/10	2/9								
Ornithodorus moubata	rhesus monkey	5/5	5/5	5/5		5/5	5/5	7/7	12/12					
	pig		4/4		4/6					4/6	2/6		2/3	
	fowl		4/4		6/6					3/6		2/6		6/6

[a] Naturally occurring mosquitos   [c] Hunger stage corresponding to the estimated period of digestion
[b] Naturally occurring flies

Reproduced from Weitz & Buxton [13]

the stomach contents could be identified up to only 24 hours after feeding (Weitz & Buxton [14]). Such variations of digestion rates under different conditions indicate the caution with which it is necessary to interpret such experiments done entirely under artificial conditions. There is, however, no indication that such differences occur among less specialized insects, such as mosquitos. The rate of digestion of blood meals of *A. aquasalis* under almost natural conditions compares well with the rates of digestion of other mosquitos studied entirely under laboratory conditions.

On the average, it seems that it would be necessary to capture mosquitos within 24 hours of feeding in order to obtain results which will be of sufficient value.

SPECIFICITY

Apart from sensitivity, reliable results of identification of the origin of blood meals can be obtained only by methods which ensure a high degree of specificity. This is a relative term which should be always carefully indicated and defined with the results of identification in order to enable the proper assessment of the value of such results. The degree of specificity of the tests which may be necessary is determined largely by the possible sources of blood meals. The procedure is thus relatively simple in such instances where the possible hosts are few and are not zoologically related. More careful methods may be required when closely related animals are available as sources of blood for the insects.

IDENTIFICATION TECHNIQUES

In regard to the conditions of sensitivity and specificity, there is no doubt that the precipitin ring test is the most convenient technique. Various other techniques have been mentioned in the literature and it may be useful to review briefly their relative values. These fall into two categories : firstly techniques which make use of the *cellular elements* of the blood meal and, secondly, methods based on the specificity of the *serum protein* of the blood.

Examination of Cellular Elements

The examination of red blood cells of blood meals is not recommended in general, as the cells usually deteriorate more rapidly than the serum proteins. There is the added danger of confusion arising over distortion or other acquired characteristics of the erythrocytes under the influence of digestion. The main difficulty, however, is concerned with the proper preservation of the blood meal. In fact, methods which depend upon the examination of blood cells are only practicable when they can be carried

out immediately after the blood meal has been obtained. It is seldom that this can be done in the field and such methods are not likely to be of much use for that reason.

(a) The direct examination of the erythrocytes under the miscroscope enables the distinction of nucleated and non-nucleated red blood cells, and as cells of reptiles are, in general, slightly larger than those of birds, it is possible, by this means, to obtain a broad idea of the relative importance of mammalian, avian or reptilian hosts for any given species of insect under consideration. Such investigations can only be regarded as a general guide on which to formulate the main plan for more careful studies. Among mammals, there is a considerable variation of the size of red blood cells and their measurements have been used as a means of identification of blood meals of tsetse flies by Lloyd et al.[7] The results obtained from such studies had their value in the absence of other means of distinguishing the large number of hosts involved, but they are indefinite and may prove somewhat misleading in the light of further studies using more reliable and delicate methods.

(b) The immunological reactions of the red blood cells of blood-meals form the basis for other methods employed for the determination of the species of origin based on the cellular components.

Recently, a rapid field test was described by Grjebine, Eyquem & Fine [5] which depends on the agglutination of the red blood cells of the insect meal by the action of various immune sera. For the reasons stated above, this procedure is not to be recommended; the other disadvantages to be considered are the difficulties of preparing truly specific immune sera and the practical impossibility of preparing such antisera against the cells of the non-domesticated species in view of the great obstacles in the collection and preservation of red cells of wild species for immunizing rabbits. This technique has also a very low sensitivity in comparison with the results obtainable by the precipitin test, which is far superior in all respects.

Examination of the Serum Components of the Blood Meal

Precipitin test

Nuttall's classical work showed that the antigenic relationship of the sera derived from animals, as demonstrated by the precipitation test, broadly correlated with the zoological classification of the animals he studied. He thus formed the basis of the precipitin test for identification of blood of animals which has been used ever since by many workers for the identification of blood meals.

Technique. The test depends upon the interaction between a saline extract of the blood meal under examination and a suitable antiserum,

usually prepared from rabbits. A positive reaction is indicated by the formation of a white precipitate which is the result of the insoluble product formed under specified conditions, when antigen and antibody are united. The absence of a precipitate therefore indicates that no antigen-antibody reaction has occurred. In practice, the test is carried out in a narrow tube about 2-3 mm in diameter and the antigen (i.e., the blood meal extract) is layered over a column of antiserum. In view of the high specific gravity of the antiserum, little mixing occurs although there is some diffusion of the fluids into each other in the region of the interface. The antigen-antibody precipitate which forms in the region of the junction of the two fluids appears as a whitish " ring ". There are numerous descriptions of the standard techniques for this test (e.g., Boyd [1]). The method of Rice & Barber [11] is useful when large numbers of tests are done. A rapid method, using the multiple serological dispenser, particularly designed for the purpose, is fully described by Weitz,[13] and the outlay for the apparatus is soon justified when a very large number of tests are performed. In these laboratories, it is usual for a single technician to test 200 or 300 blood meals in a morning against possibly half a dozen antisera, by means of the multiple dispenser. A method which was devised by Hole (unpublished document) may be very suitable for testing a relatively smaller number of blood meals using a home-made apparatus. A 2-ml hyperdermic syringe is fixed vertically with the nozzle pointing downwards on to a solid wooden board. The plunger of the syringe is fitted with an extension spring and above it a wheel bearing an eccentric cam is fitted so that the thumb piece of the plunger is in contact with the cam. By the rotation of the wheel in one direction the piston is lifted smoothly, while the opposite rotation causes the plunger to move downwards. A short piece of rubber tubing is connected to the nozzle of the syringe and a narrow glass tube (2-3 mm internal diameter × 2.5-4 cm long) is forced into the rubber connectors. It is a great advantage to have the glass tube drawn to a fine capillary at one end. By dipping the open end of the capillary into the fluid, first the antigen and then the antiserum is drawn into the tube immediately below it. A little air is finally drawn up so as to move the two fluids up the middle of the tube and the capillary end is sealed with a small flame. The tube is removed and stood in a plasticine rack. With a little practice it is possible to test quite rapidly a fairly large number of blood meals this way.

Blood meals

(*a*) The collection of blood meals should be done with scrupulous attention to prevent the contamination of one blood-meal with another. The stomach contents are obtained by laying the gorged insect on a filter-paper of good quality on the site where it is intended to make the smear.

The insect is then roughly dissected with a pair of needles so that the abdominal contents are squeezed on to the filter-paper, the remaining carcass of the insect being discarded. With very freshly fed insects there is no difficulty in obtaining a good smear on to filter paper, but with poorly fed insects it may be necessary to squeeze out all the stomach contents with a dissecting needle. To avoid carrying over small amounts of serum from one smear to the other, it is most important that the needles or other instruments used for the dissection should not be re-used for another insect, unless they have been thoroughly cleaned. The smears should be made along the periphery of the filter-paper about 1 cm from edge. In this way, about a dozen smears can be accommodated on 10-cm diameter filter-paper. The smears should be allowed to dry thoroughly, preferably in a dessicator. When sent by post or packed otherwise, the filter-papers containing the smears should be interleaved with non-absorbent circles of paper and the whole contents packed in a waterproof container. The method of recording the smears is obviously a matter of individual choice, but there should be as little writing as possible on the filter-paper itself. A useful way is to divide the filter-paper into suitably numbered sectors, each filter-paper bearing a specific code number. The details and records can be set out on key sheets on to which the result of blood-meal tests can be entered directly.

(b) The extract of the blood meal is made by cutting out the blood spot from the filter-paper and placing it into a properly labelled test-tube of suitable size (e.g., 10 mm × 5 mm) to which is added a quantity of normal saline. The volume of saline added is dependent upon the number of tests which are to be done on the extract as well as on the quality of the blood smear. The object is to use as much saline as is possible without causing the dilution of the antigenic material beyond the sensitivity of the method of testing. It is possible, with some experience of the appearance of blood smears from any species of insect, to judge the permissible level of dilution. Mosquito extracts, for example, can usually be diluted by adding 0.5 ml of saline to the smear, providing that some blood is still visible. This amount of extract is sufficient for approximately 10 precipitin tests. When poor feeds are encountered it is usual to reduce the amount of saline to a minimum. Thus, if 5 precipitin tests are required, the extract is made by adding 0.25 ml of saline, which should be enough with economical use although it is unlikely that material will be available for re-testing as a check. After the saline has been added to the smears, the tubes are kept at 4°C for 6-10 hours before testing. In practice, the extracts are made at the end of the day and are ready to be tested the next morning. The number of tests which can be made in a single day will determine the number of extracts to be prepared and such a procedure will avoid keeping extracts for too long periods, as when the blood smears have been dissolved, deterioration of the antigen is relatively rapid. It is advisable to mix the contents

of the tube before testing by very gentle rocking; too vigorous shaking will cause particulate matter to become suspended and such extracts when tested may give rise to false positive readings as the particles tend to drop from the column of antigen and rest on top of the antiserum, thus simulating the presence of a single precipitate at the junction of the two fluids. Heavy contaminations of the extracts with micro-organisms, particularly moulds, may have the same effect. When extracts are cloudy, it is best to filter them before testing in order to avoid any possibility of confusion. This can be done by means of centrifugal membrane filters of small diameter which prevent any loss of material.

Preparation of antisera

The careful preparation of the antisera for the precipitin test is of the utmost importance. The procedure is described in detail by Weitz [12] and is summarized here for convenience.

Antigen for immunization. It has been found that the method described by Proom [10] is probably the most satisfactory way of preparing the antigen for immunization of rabbits. Other immunizing adjuvants, e.g., Freund's adjuvant,[3] have not been so successful, and it also takes longer to immunize rabbits satisfactorily in this way. To prepare alum precipitated serum (A.P.S.) it is preferable to use relatively fresh serum if possible, although good results are obtained from sera which have previously been kept dried from the frozen state. Ten ml of serum are diluted with 30 ml of distilled water and then 33 ml of a 10% solution of potash alum is added. The pH must be adjusted to 6.5 with 5N, NaOH solution to precipitate the maximum amount of antigenic protein. The precipitate thus formed is then collected by centrifugation and washed twice in normal saline containing a concentration of 1/10 000 thiomersalate and finally re-suspended in 20 ml thiomersalate saline.

Immunization of rabbits. Healthy rabbits are injected with a dose of 5.0 ml of A.P.S. into each hind limb, the dose being repeated 12 days later. Ten to twelve days after the last injection, the rabbit serum is tested for its titre of sensitivity with the homologous antigen and if satisfactory (i.e., 1/20 000 at least) 50 ml of blood is taken from the heart. Repeated doses of 5.0 ml into each leg may be given about a week after bleeding, the procedure being repeated as long as the titre of the antiserum is satisfactory, 50 ml of blood being drawn at each immunization. The serum is separated from the blood after it has been allowed to clot, and is stored at −10°C without preservative.

Standardization and absorption of antisera

When sufficient antiserum of one kind has been accumulated, a pool is made and the titres at which the homologous and heterologous sera react

with it are determined. In the case of an antiserum against the serum of a mammalian species, it will be found that cross reactions occur at very high titres with sera from most mammals. Similarly, antisera prepared against the serum of a bird will generally react with many avian sera. The antibodies responsible for these cross reactions are removed by absorption. The following example describes the general procedure for absorption of anti-human serum.

A mixture of equal parts of the serum of ox, horse, sheep, dog and pig is made and a series of twofold dilutions in saline (from 1/2 to 1/128) is prepared. A series of 8 small test-tubes each containing an equal volume (0.5 ml) of antiserum is also prepared and to each of these tubes is added a tenth of the volume (0.05 ml) of the mixture of heterologous sera, the first tube receiving the undiluted mixture, the second tube the 1/2 dilution, and so on. The final concentration of the antigen mixture is thus a tenth of the original dilution, as shown in Table II.

TABLE II. TECHNIQUE FOR PRELIMINARY ABSORPTION OF ANTISERA

Number of test-tube	1	2	3	4	5	6	7	8
Undiluted antiserum (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution of absorbing sera	neat	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Volume of absorbing sera added (ml)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Final antigen-antiserum ratios	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280

The tubes are well mixed and stored at 4°C overnight. They are then centrifuged to remove all the precipitate and the supernatant fluid is tested for the presence or absence of heterologous antibodies by the precipitin ring method, using 1/10 and 1/100 dilution of each serum in turn. Table III illustrates a typical result.

In the example shown in Table III, all the heterologous antibodies have been removed in tube 3 containing 1 part of the antigen mixture to 40 parts of antiserum. In tube 4, containing a ratio of 1 part of antigen mixture to 80 parts of antiserum, some antibody against ox serum and perhaps against horse serum remains.

Bulk absorption

In practice, from such a result, it would be usual to absorb the main bulk of antiserum with 1 part of the antigen mixture to 100 parts of anti-serum. The mixtures are well shaken and left for at least 24 hours at 4°C. After this time, a sample of the bulk is taken and is filtered through a

TABLE III. RESULTS FROM TESTING SUPERNATENT AFTER PRECIPITATION OF VARIOUS ANTIGEN-ANTISERUM MIXTURES

Test-tube number	1		2		3		4		5		6		7		8	
Antigen-antiserum ratios	1:10		1:20		1:40		1:80		1:160		1:320		1:640		1:1280	
Serum dilution	$\frac{1}{100}$	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{10}$
Ox	—	—	—	—	—	—	+	++	+++	+++	+++	+++	+++	+++	+++	+++
Horse	—	—	—	—	—	—	—	+	++	+++	+++	+++	+++	+++	+++	+++
Pig	—	—	—	—	—	—	—	—	—	+	++	+++	+++	+++	+++	+++
Sheep	—	—	—	—	—	—	—	—	+	++	+++	+++	+++	+++	+++	+++
Dog	—	—	—	—	—	—	—	—	—	—	—	+++	+++	+++	+++	+++

+ to + + + = presence of precipitate when tested by the precipitin ring test.

clarifying asbestos pad. The filtrate is then tested with a dilution of 1/10 and 1/100 of each of the heterologous sera by the ring test. In this example, it would be likely that a positive reaction would be obtained with ox serum in which case a small amount of ox serum (e.g., 1 part to 1000 parts of antiserum) is added to the bulk which is shaken and left for a further 24 hours at 4°C. A re-test will show if all the antibodies reacting with ox serum have now been absorbed. If no positive reaction occurs when a filtered sample of the absorbed bulk is tested with dilutions of each of the heterologous sera at a dilution of 1/10 and 1/100, the whole bulk is then filtered through a Seitz EK sterilizing pad and the titre at which the homologous antigen (human serum in this example) will react by the ring test, is finally determined. There is usually no appreciable drop in homologous titre following absorption of the antiserum providing that the amount of the heterologous antigens added was the minimum required for complete absorption of the heterologous antibodies.

Storage of the antiserum

The sterile antiserum is now ready for immediate use. It is inadvisable to add any preservatives as these cause precipitation and resulting cloudiness of the antiserum. In order to keep the antiserum sterile, it can be dispensed into sterile ampoules of suitable size and kept at −10°C. If storage over a period longer than one or two months is necessary, it is ideal to dry the antiserum from the frozen state. Beforehand, it is essential to remove the lipoids which would precipitate at the low temperature at which the antiserum is subjected during the drying process, and which would cause opalescence of the antiserum when reconstituted. It is done by McFarlane's method [8] of mixing the antiserum with ½ volume of ether and freezing the mixture at −25°C. When thawed, the ether is syphoned off and the antiserum refiltered to remove the precipitated lipoids. Antisera treated this way, and subsequently dried from the frozen state, have been kept for 5 years at least without any change in the characteristics of the antiserum. There is the added advantage that a crystal clear antiserum is obtained when reconstituted with distilled water.

Inhibition test

While the precipitin test is sufficiently specific to distinguish the sera of man and domestic animals, reactions occur with sera of closely related animals. For instance, absorbed antihuman serum which fails to give any reaction with the sera of any of the domestic animals, gives rise to cross-reactions when tested with the sera of many of the primates. It has not yet been found possible to absorb the antibodies responsible for these cross-reactions without causing a very appreciable drop in the titre for the homologous serum. Similarly, antipig serum fails to distinguish warthog, bush-pig or domestic pig; horse, zebra, mule and donkey sera all cross react with

antihorse serum; while an even wider range of bovid sera react with anti-ox or antisheep sera.

Although these cross-reactions are of no importance in the study of " domestic insects " which occur in areas where the tests are limited to man or domestic animals, the study of the feeding habits of " wild insects " may demand identification of hosts which are closely related, as, for example, in the study of forest mosquitos, tsetse flies and other vectors which may draw their blood supply from a wide range of animals, some of which are closely related to each other.

For this purpose, the inhibition of agglutination test was devised as a new means of identification. This is a somewhat elaborate test which is extremely sensitive and is only usable with comparatively good quality feeds. A brief report has already been made of its use by Weitz & Jackson [14] and as it is not commonly required for the identification of blood-meals of malaria mosquitos only a brief reference to it will be made here. The complete details of the technique and its appreciation are the subject of a communication now under preparation.

The principle of the inhibition test is based on the technique described by Boyden [2] which uses normal red blood cells which are coated with tannic acid and which are then capable of being sensitized with soluble antigens. In this way it is possible to coat the cells with a selected serum protein antigen and such cells will be agglutinated by an antiserum prepared against the protein with which the red blood cells have been sensitized.

For the brief description of the essential parts of this test, an example is given of the procedure adopted for the identification of the origin of a blood-smear which gave a positive reaction when tested with antihuman serum by the precipitin test and which might therefore have originated from man, baboon or monkey.

Firstly, a test for the presence of human serum is applied. Normal red blood cells of rabbit are treated with tannic acid and are then sensitized with normal human serum These are capable of being agglutinated by antihuman serum at a suitable dilution. The agglutination can be inhibited by treating the previously selected concentration of antiserum with varying dilutions of human serum up to approximately 1/4000 to 1/8000. At the optimum dilution of antiserum, the inhibition of agglutination of sensitized erythrocytes is species specific, i.e., neither baboon nor monkey sera, even at a high concentration, are able to cause inhibition. It follows that if the antiserum at the selected dilution is mixed with a blood-meal extract which contains human blood, the agglutination of the red blood cells sensitized with human serum will be inhibited. If the blood meal contains baboon or monkey or any other blood, no inhibition of the antiserum is evident.

The specific inhibition will occur only if the antiserum dilution is carefully selected. The use of a higher concentration of antiserum than the optimum will result in a decrease in the sensitivity, i.e., a greater concen-

tration of human serum will be necessary to inhibit the reaction. This is an important factor, as a blood meal may not always contain sufficient serum to cause inhibition of the antiserum. If the concentration of the antiserum is less than the optimum, the sensitivity is increased considerably at the expense of specificity so that under such conditions, human serum will inhibit the reaction at a dilution greater than 1/4000 to 1/8000, but at the same time baboon or monkey sera will also inhibit the agglutinating properties of the antiserum for human sensitized cells. The determination of the optimum concentration of the antiserum which will be inhibited with maximum sensitivity and specificity is very critical and varies considerably for every batch of sensitized red blood cells. Table IV shows the results obtained when tanned red blood cells, sensitized with human serum, are added to various concentrations of antiserum which have been mixed previously with varying dilutions of human, baboon and monkey serum. The optimum concentrations of the antiserum in this case is a dilution of 1/20 000 of antiserum. At this concentration, the highest dilution of human serum which inhibits the reaction is 1/8000 and no inhibition of agglutination is caused by even high concentrations of baboon or monkey sera. With the dilution of 1/25 000 of antiserum, inhibition is caused by both baboon and monkey sera, while the dilution of 1/15 000 of antiserum, although specifically inhibited, is not sufficiently sensitive.

TABLE IV. SPECIFIC ABSORPTION OF AGGLUTININS AGAINST TANNED RED BLOOD CELLS SENSITIZED WITH HUMAN SERUM

Dilution of antihuman serum	Inhibiting serum	Dilution of inhibiting serum								Controls	
		$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{4000}$	$\frac{1}{8000}$	$\frac{1}{16\,000}$	$\frac{1}{32\,000}$	anti-sera	antigen
1/25 000	man	—	—	—	—	—	—	—	++	+++	—
	baboon	—	—	—	—	++	+++	+++	+++	+++	—
	monkey	—	—	—	—	+	++	+++	+++	+++	—
1/20 000	man	—	—	—	—	—	—	++	+++	+++	—
	baboon	++	+++	+++	+++	+++	+++	+++	+++	+++	—
	monkey	+	+++	+++	+++	+++	+++	+++	+++	+++	—
1/15 000	man	—	—	—	—	+++	+++	+++	+++	+++	—
	baboon	+++	+++	+++	+++	+++	+++	+++	+++	+++	—
	monkey	+++	+++	+++	+++	+++	+++	+++	+++	+++	—

+ to +++ = agglutination of sensitized red blood cells often occurring after antihuman serum has been inhibited by various concentrations of sera of man, baboon or monkey.

It follows that providing the blood meal extract contains at least the equivalent of a dilution of 1/8000 of human serum, it will cause the inhibition of the antihuman serum at a 1/20 000 dilution when mixed with it, as is shown by the subsequent addition of tanned erythrocytes sensitized with normal human serum. If the blood meal contained baboon or monkey serum, no inhibition is caused and agglutination will occur at this concentration of antiserum. The inhibition is completely reversible in the sense that an antibaboon serum is similarly specifically inhibited by baboon serum when tested with tanned red blood cells sensitized with baboon serum, or a system using antimonkey serum and cells sensitized with monkey serum is inhibited specifically by monkey serum. For the test of the origin of the blood meal, all these systems are used and the results obtained confirm each other, as indicated in Table V. As the method is somewhat complicated and technical details are numerous, it would be beyond the scope of this paper to describe this technique any further. A full report will be published later.

TABLE V. IDENTIFICATION OF AN UNKNOWN SERUM (MAN, BABOON OR MONKEY)

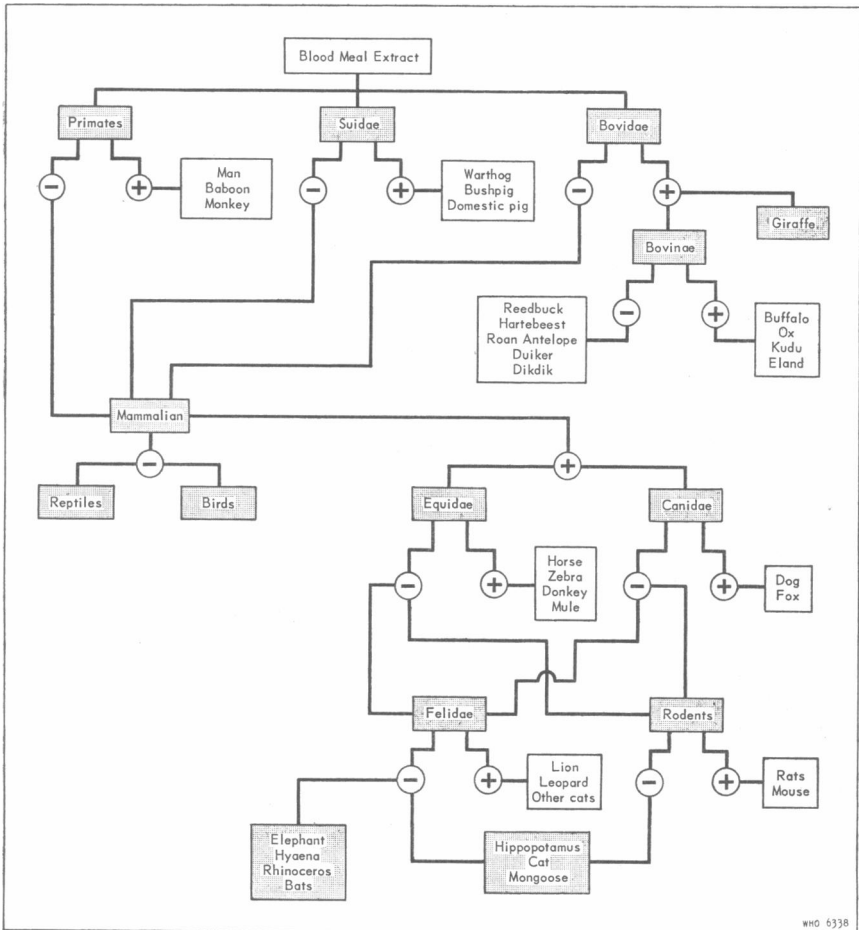| Testing system		Agglutination after inhibition with the unknown serum	Identity of unknown serum
Antiserum	Red blood cells sensitized with the serum of		
antiman	man	—	man
		+	baboon or monkey
antibaboon	baboon	—	baboon
		+	man or monkey
antimonkey	monkey	—	monkey
		+	man or baboon

General Summary of the Procedure for Identification Tests

If the range of suspected animals is large, it is necessary to economize material by screening the blood meals first of all for the most likely hosts. The procedure described here is usually adopted for identification of blood meals of tsetse flies which may serve as an example of the most elaborate procedure likely to be required.

After extraction, the blood meals are tested for the most likely group of hosts by the precipitin test using antihuman, antipig and antibovid serum. The latter is prepared by inoculating rabbits with a mixture of antisera so that it will react with all the species of bovids involved, but not with sera from species other than Bovidae. The feeds which are found positive to antihuman serum will then be tested by the inhibition test for man, baboon and monkey.

Similarly, feeds reacting with antipig serum are distinguished into wart-hog or bush-pig feeds by the inhibition test, and the feeds reacting with the general antibovid serum are sorted out for the species of Bovdae by the inhibition technique; this may require a large number of tests as the variety of bovids which are possible hosts may amount to at least 5 or 6 different species. If larger numbers of species are available as hosts, it is necessary to test the feeds for further groups of bovids by the precipitin test (e.g., for

FIG. 1. PROCEDURE FOR THE IDENTIFICATION OF BLOOD MEALS

Blood Meal Extract

Primates — (−) / (+) — Man / Baboon / Monkey

Suidae — (−) / (+) — Warthog / Bushpig / Domestic pig

Bovidae — (−) / (+) — Giraffe

Bovinae — (−) Reedbuck / Hartebeest / Roan Antelope / Duiker / Dikdik / (+) — Buffalo / Ox / Kudu / Eland

Mammalian — (−) Reptiles / Birds

(+) Equidae — (−) / (+) — Horse / Zebra / Donkey / Mule

Canidae — (−) / (+) — Dog / Fox

Felidae — (−) / (+) — Lion / Leopard / Other cats

Rodents — (−) / (+) — Rats / Mouse

Elephant / Hyaena / Rhinoceros / Bats

Hippopotamus / Cat / Mongoose

WHO 6338

White boxes = inhibition test
Shaded boxes = precipitin test

In order to economize material, the procedure outlined in this figure can usefully be followed. First test the blood meal with antihuman, antipig and antibovid serum. If the result is negative, test further with antimammalian and antiavian serum. If the result is positive, test for further identification with specific sera in the mammalian groups indicated in the chart; if it is negative, the sera are either of reptilian origin or unsuitable for testing.

presence of ox or buffalo), the individual groups are further subdivided into species of origin by the inhibition test. This procedure varies according to the circumstances, but the aim is to conserve as much material as possible for the species differentiation by the inhibition test.

Having thus sorted feeds which originate from the Primates, the Suidae or the Bovidae, a proportion of feeds is left unidentified. At this stage, if avian or reptilian feeds are suspected to be of some importance, the remaining blood meals are tested with a general antimammalian, an antiavian serum and an antireptilian serum which react with the sera of all mammals, birds and reptiles respectively. Feeds which are positive with antimammalian serum are then tested for mammalian groups by precipitin tests, e.g., the Equidae or the Canidae and if no positive reactions are observed, the blood meal is tested for as many species as possible by the precipitin test as, for example, for elephant, rhinoceros, hyaena, etc. As these animals have no relatives, the antisera are in practice species specific.

The feeds which fail to react with antimammalian, antiavian sera, or antireptilian sera must be presumed to be unsuitable for testing, since the titre of these general antisera is about the same as for other antisera. The procedure is diagrammatically represented in Fig. 1. This protocol ensures that the identification of the most likely group of species is arrived at as soon as possible in order to conserve material for the inhibition tests which may be somewhat extravagant on the amount of material used. Approximately 0.05 ml of extract is used for the precipitin test and about 0.15 ml, for each species, for the test by inhibition. Thus, if a feed is derived in fact from dikdik, the amount of material used before it is identified would be 3 × 0.05 ml for the group tests and 5 × 0.15 ml for the species test, i.e., 0.80 ml of the extract, leaving about 0.2 ml for confirmatory tests if required. The test with antimammalian serum is done at an early stage for two reasons: (1) It confirms whether the feed contains sufficient antigenic material at all; if this was left till a later stage it might well be that no material was left for a test with antimammalian, antiavian, and anti-reptilian sera and some doubt would ensue as to whether the feed was in fact too poor for identification or whether the suitable test for the species of origin had not been applied by the time the material was completely used up. (2) If, at an early stage, a feed is not found to contain mammalian blood, an obvious saving of material and time is achieved by avoiding unnecessary tests for mammalian hosts.

RÉSUMÉ

Les tests d'identification du sang ingéré par les arthropodes piqueurs doivent être assez sensibles pour révéler la présence et la nature de sang déjà partiellement digéré et assez spécifiques pour indiquer l'espèce animale dont il provient. Le contenu stomacal ne renferme en effet souvent qu'une faible proportion de protéines identifiables et durant un temps très court. Il est difficile de capturer les insectes sitôt après qu'ils se sont gorgés, en particulier ceux qui se reposent à l'extérieur des habitations. La vitesse de digestion varie selon que l'arthropode est maintenu en captivité ou laissé en liberté. Le contenu stomacal de 95%-100% de glossines gorgées, maintenues en captivité, était encore identifiable après trois jours; il ne l'était que chez 5%-10% de ces insectes (marqués) remis en liberté après piqûre et capturés à nouveau. On interprétera donc avec grande prudence les résultats obtenus en laboratoire. D'une façon générale, il est souhaitable de capturer les moustiques au plus tard 24 heures après le repas de sang si l'on veut être assuré de la validité des résultats.

Deux types de méthodes d'examen ont été employées: les unes fondées sur l'examen des éléments cellulaires, les autres sur la spécificité des protéines sériques.

L'examen microscopique direct des érythrocytes permet de distinguer avec une certaine sûreté les sangs d'oiseaux, de reptiles et de mammifères, mais la différenciation des hématies de mammifères les unes des autres n'est qu'approximative.

Les réactions immunologiques des corpuscules sanguins trouvés dans le contenu stomacal sont à la base des autres techniques d'identification. La méthode des précipitines, supérieure à toutes les autres, est fondée sur la correspondance entre le rapport antigénique des sérums, tel qu'il apparaît dans le test de précipitation, et la classification zoologique.

Le test consiste en une réaction entre un extrait du repas de sang dans le soluté salin et un antisérum convenable, préparé sur le lapin. La précipitation indique une combinaison antigène-anticorps.

Une description détaillée de la technique du test est donnée par l'auteur. Elle comporte le prélèvement du repas et sa fixation sur papier filtre, l'addition de soluté salin, la préparation des antisérums sur le lapin, la normalisation des titres et l'absorption des antisérums donnant des réactions croisées, enfin le test d'inhibition utilisé lorsqu'il s'agit de préciser l'espèce animale, au sein d'un groupe animal donnant des réactions croisées. C'est le cas en particulier pour les insectes des forêts, les glossines et d'autres vecteurs, qui piquent un grand nombre d'espèces, souvent apparentées.

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