ant endemic foci are confined to the Gulf coast, Louisiana furnishing by far the largest part.

The number of lepers in the country known and reported by health officers is under 150. The true number probably is double this at least. To show how chaotic is our information on this point, I may say that at a recent hearing before a Senate committee in Washington the estimates of the number of lepers in the United States varied from 150 to 2400.

Original Articles.

SOME EXPERIENCE BEARING ON THE MEDICO-LEGAL VALUE OF THE PRECIPITIN TEST FOR HUMAN BLOOD.*

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FOREWORD.

FOURTEEN years ago† Professor Edward S. Wood, of honored memory, read a paper before this Society entitled "The Serum Test for Human Blood," in which he described the so-called precipitin test of Uhlenhuth, and states: "By our old methods (without this recent test) we were able to testify that a certain blood-stain gave results on measuring the red blood cells obtained from the stain, which showed that the blood-stain was consistent with its having been made by human blood, and, further than that I do not know that any expert has ever testified. But now if we obtain a positive result by this serum test, we can say that the stain contains human blood serum, and does not contain that of any other animal except some species of monkey."

The conclusion Dr. Wood then announced became the accepted view as to the value and reliability of the test throughout this community, both among physicians and officers of the law, with the additional inference, supported by certain of our text-book writers, that the age of the stain did not materially increase the difficulties or detract from the reliability of the reaction.

This paper is based on:

1. An experience entailing a disappointment.
2. A series of laboratory efforts to explain or supplement the experience.

* Read, in slightly different form, before the Massachusetts Medico-Legal Society, February 2, 1916.

† February 4, 1902.

THE EXPERIENCE.

My* particular interest in this test was aroused by an important murder case which occurred in our district, and for which a young man was tried by the Superior Criminal Court, at Worcester, Mass. The story of the case is briefly this: The victim was a peddler of dry goods and furnishings, who drove a cart through some of the mill towns in the southern part of the 11th Worcester District. About 11.30 p.m. on a certain date, the night officer in A—— met the team jogging slowly toward A—— from the direction of B——. Not receiving an answer to his salutation, he investigated, and found the body of the owner, head down, in front of the seat, legs up over the seat, wrapped in blankets, and having a wound in the front of the chest. The autopsy showed that he had been shot from behind with a shotgun, at close range. His hat was trampled with hay, chaff and manure, and the trail of the wagon in the snow led to the mill hamlet of C——, seven miles south, where the victim had been and where his movements were traced up to 8.30 p.m. Next day there were found in the stable where he customarily put up, especially in the stall opposite the one his horse usually occupied, and scattered about the floor adjacent, many blood stains, mostly matted with straw, bran or chaff, and some also on the middle plank of the stall floor. These were variously explained as due to the killing of fowl, the slaughter of pigs (usually done in the adjoining wash-room), and by the discharges of a bull with "black water," which had been kept in that stall two weeks previously. Most of the blood spots were moist when found. Later a gun capable of inflicting the wound was found broken, and the portions hidden in different places within the barn. Still later, the watch of the murdered man was found carefully hidden in the house, and on the same floor as the room occupied by the young man who was in charge of the stable at the time the murder must have happened, and who had the only key to the room where the gun was customarily kept, not otherwise accounted for.

By order of the District Attorney, the blood stains were turned over to Prof. William F. Whitney for identification. A few weeks later, however, Prof. Whitney advised us that he was awaiting a serum from Germany, and feared that it might not arrive because of the difficulties of communication with that country, and invited me to prepare a serum.

Accordingly I began the immunization of four healthy rabbits by the technic of Dr. Paul Uhlenhuth, as described and exemplified in his monograph, kindly loaned me by Prof. Whitney, varying only and very slightly the intervals of injection, as the exigencies of obtaining the necessary blood compelled, but adhering very closely to the optimum of 2.5 cc., intravenously, every five days for three doses, bleeding on the seventh

* E. L. H.

day, after the last dose. After a much longer period than this and by repeated injections, I finally produced an active serum (titer 1-5000) just a day before Dr. Whitney received a supply from Germany, which gave a higher titer (1-10000 feebly) but reacted less promptly and with less definiteness than mine in the lower dilutions.

Armed with these sera, Prof. Whitney and I,* carefully watched in the interests of the defence by Prof. A. W. Balch, proceeded with the tests as follows:

1. Ascertained the titers as above.
2. Ascertained the specificity against horse, beef, pig and sheep blood, 1-200 dilution, finding both negative to these species.
3. Tested solutions from dried blood stains, made with .85 saline solution, carefully centrifugated to clearness and diluted so that all gave a cloud with nitric acid, corresponding as nearly as possible to 1-1000 solution of fresh human serum, as follows:

(a) Blood from the victim's shirt now about three months old.

(b) Old blood stain on dress of another murder case about nine months old.

(c) Blood clot from stable, using best specimen, found in moist state and practically free from straw.

(d) Human blood on gauze pad, dried in laboratory six weeks.

There was absolutely no reaction in any of these tests, although both sera reacted promptly and well with fresh human serum.

Drs. Whitney and Balch reported to their respective attorneys failure to identify the blood beyond the general statement that it was of mammalian origin.

The accused was acquitted.

After the trial we secured the blood-stained plank and a scant specimen of the bloody straw, and began a further study of the method.

THE LABORATORY STUDY.

(a) *The Preparation of the Immune Sera.*

We have prepared our sera by the immunization of healthy rabbits, chiefly by the intravenous injections of fresh, sterile, human serum obtained: (1) from bloods sent to the laboratory for Wassermann tests for syphilis, (2) stripings from the placental ends of the umbilical cords in the maternity ward, drawn immediately after severing the same, (3) occasional bleedings for therapeutic purposes. The bloods aseptically drawn were allowed to clot over night, after loosening the initial early clot from the walls of the container with a platinum wire, the resulting clear serum being pipetted off. Rarely a slight admixture of corpuscles had to be separated by centrifugation, although in certain cases this was not done, as Hektoen states that by retaining corpuscular elements an anti-serum of greater value for medico-legal work is obtained; (4) hydrocele and pleuritic fluid were

made use of on occasion to supplement our supply of human serum, but proved to have less active antigenic properties.

As to the matter of dosage and intervals between injections, we find that the chief authorities agree only in a general way. With one exception, we have started the rabbits according to the method advised by Uhlenhuth, which is to inject two or three cc. into the ear veins, every 5 days for 3 doses, bleeding for trial on the seventh after the last dose, and daily for two or three days, bleeding in quantity when the trials show a valuable anti-serum. We did not follow his advice to kill the animals and take all possible blood when a satisfactory serum had developed, for the reason that we never found one to give the 1-20,000 titer, that he stipulates, so always continued the immunization, hoping to raise the value. When continued immunization seemed indicated, it was our plan to give a new series, with the doses and intervals as before, and this sometimes succeeded on the fourth, or more, trials. (See Nos. 2 and 12, second series.) Sometimes after a rest of several weeks, a resumption of injections would produce a better serum than a former series had done. (See No. 8, 2d series.)

The one exception was made in an effort to apply the rapid method of Fornet and Muller, as recommended by L. Hektoen. This rabbit (No. 11, 2d series) received by the intraperitoneal route three doses of defibrinated blood on consecutive days of 5 cc., 10 cc., and 15 cc., respectively, and was bled every other day thereafter by aspirating the ear vein for a test amount, 2 cc.; the reaction of immunity attaining its high point on the 14th day after the last dose, remaining there for about five days, then receding.

Our method of obtaining the test portions of blood by aspirating the ear vein, we have not noted in other works. It requires considerable dexterity of manipulation, but is inoffensive to the rabbit, and does not maim the ear.

We obtained the blood in quantity when the immunization had reached a satisfactory point, as indicated by the test titrations, by means of cardiac aspiration. This is well borne by the rabbit when correctly done, yields 15 to 30 cc., and has the great advantage of asepsis, and the lesser of preserving the animal for further use.

The blood so obtained is driven out of the syringe into a sterile petri dish slanted so that the clot will form at one side. When the clot has fairly formed it is placed in the ice-box, slanted in the opposite direction. Next day the clear serum is removed with a sterile pipette, titrated, tested for specificity, and, if it is to be stored, is sealed in glass ampoules, and thereafter stored in the ice-box with all light excluded, in the upright position, to allow corpuscles to settle, if any remain (Nuttall). Usually the animals are starved 24 hours before bleeding. (Uhlenhuth, rule to avoid opalescence.)

* R. L. H.

The chief difficulty encountered during the progress of the immunization was the rather frequent death of a rabbit from anaphylactic shock. The third dose by the Uhlenhuth method was usually the first to be dreaded, but we lost most after several series of injections had been administered, the symptoms being dyspnea, paralysis of the hind quarter, then forequarter, then respiratory failure, and all in a period of 5 to 10 minutes. In several instances they would rally after a period of dyspnea, or even after the paralysis of the hind quarters, and be hopping about as usual after a half hour.

Altogether we immunized fourteen rabbits, of which seven gave at some period a workable serum (*i.e.* 1-1000 to 1-2000), three gave sera of fairly high value (1-5000 to 1-10,000). Of the fatalities, 4 died before producing a serum of any value, three of these being from anaphylactic shock, and one from abuse by other rabbits. Of the other three, one died of shock after producing our best serum, two died from heart aspiration, after producing workable sera. (See Tables 1 and 2.)

TABLE 1.

FIRST SERIES	IMMUNIZED BY	BEST TITER	RESULT
1	Pleuritic fluid	Opalescent	Died from bleed-
	H. blood serum	Very low	ing
2	Human serum	1/1000+	Sick—killed
3	Pleuritic fluid and H. serum	1/500++	See series No. 2
4	Human serum	1/5000+	See series No. 2
5	Human serum	1/1000+	Retrogression
			See series No. 2
7	Human serum	1/500+	Died from bleeding
8	Human serum	1/500++	See series No. 2
9	Human serum	1/1000+	
10	Pig serum		Died

Table 1 shows results of first series of immunization experiments.

TABLE 2.

SECOND SERIES	IMMUNIZED BY	TITER	RESULT
1	Hydrocele fluid and H. serum	1/2000+	Died after bleeding
2	Same	1/10,000±	Still living
3	Beef serum	1/500+	Died. Serum infected
4	Pig serum	1/500+	Died—anaphylaxis
4	Pig serum	0	Died—infection
5*	Human serum	1/2000±	Died—anaphylaxis
6†	Human serum	0	Died—anaphylaxis
7	Beef serum	1/100±	Died—infection
8‡	Hydrocele fluid and H. serum	1/1000+	Still living
9§	Hydrocele fluid and H. serum	1/5000+	Died—anaphylaxis
10	Defibrinated H. blood—Hektoen method	1/1000+	Still living
12	Sheep serum	1/4000+	Still living
13	Pig serum		Not completed
14	Human serum	1/200+	Still living

Table 2 shows results of second series of immunization experiments. Pig and beef blood from slaughter house always contaminated. Sheep serum, aseptic.

* No. 4 in First Series.

† No. 3 in First Series.

‡ No. 5 in First Series.

§ No. 8 in First Series.

None of our rabbits gave sera of the high value recommended by Uhlenhuth (*i.e.* titer of 1-20,000). Comparing these results with those of other workers, we find that in Uhlenhuth's published protocols of 13 rabbits immunized with human serum, five were worthless, two reached the 1-20,000 point, but retrograded; five gave 1-20,000 serum, and one gave a titer of 1-200,000, these last six being killed, and the sera preserved.

Some years later in his Harben lecture (1911) Uhlenhuth made the statement that it is not uncommon to find but one rabbit in a series of ten capable of producing serum of the potency he requires.

Hektoen immunized nine rabbits by the rapid method, which he favors, with these results: One was refractory, four gave workable (1-2000 or under), two 1-10,000 to 1-12,000, respectively, two gave 1-20,000, his titers being read after standing one hour at room temperature.

Many writers do not specify their standards, but refer to their sera as strong or weak. Wassermann and Schütze use a different method of standardizing, but utilize relatively weak sera, warning against too high potency as tending to give group reactions. Muir and Martin say, "With the serum of rabbits vs. man .05 cc. precipitation is distinct with .001 cc. of human serum, almost absent with .0001 cc." Hektoen points out, and Uhlenhuth's protocols reveal that it is a matter of greater difficulty to obtain a rabbit anti-human serum of high potency than is the case with that of lower animals. This is also in keeping with the experience of many in preparing anti-human amboceptor for the Noguchi modification of the Wassermann test, and is the chief reason that it is not in more general use at present. Nuttall and Graham-Smith used the proportions of 1 part immune serum to 20 parts of solution of blood to be tested in their experimental work.

Granting the desirability of sera of high potency, especially for the important work of identifying medico-legal specimens, we do not feel that the conclusions to be drawn from the experiments about to be detailed are vitiated from having been made with sera of relatively low titer, though regretting that they are less far-reaching in their bearing upon the subject than they might otherwise have been.

More especially is this experience of value since it is the purpose of this paper to emphasize the difficulties and uncertainties of the method, that in cases where the circumstantial basis depends upon the identification of blood stains, they may be anticipated, and their possible consequences minimized by the exercise of suitable precautions on the part of the medical examiner.

In carrying out our tests we have followed the technic described by Uhlenhuth in his monograph, using always 1 cc. of the test solution of various strengths, and adding .1 cc. of the anti-

serum, except in some instances, where we used .05 or .06 cc. for reasons of economy. The tabulated experiments are with the former amount. The test solutions which he prescribes should be made to as near 1-1000 as possible, for the medico-legal test of a stain, and the controls are: (1) the test solution against normal rabbit serum, (2) fresh human serum against the anti-serum, (3) serum of some lower animal 1-200 against the anti-serum, (4) of some other animal ditto, (5) 0.85 saline, (6) an extract of the fabric or material from which the stain is taken.

PRECIPITIN TEST.

Typical Reaction (after settling 24 hours). All negative save No. 1 and No. 3, the test serum and known human serum, respectively; all other controls clear.

For the experimental work we have used more concentrated test solutions, as seemed more suitable to the titers of our anti-sera, viz: 1-100 or 1-200 as advised by Nuttall.

For the purpose of showing the intensity of our reactions we have adapted the method in common use for recording Wassermann tests, using a three plus (+++) to indicate a strong, two plus (++) a fair, one plus (+) a slight but definite, and a plus or minus ($\pm$) a very slight reaction.

(b) Trial Applications of the Test.

For nearly a year, in anticipation of the time when we should be provided with adequate sera, we saved blood material for the purpose of studying the efficiency and reliability of the method, particularly as regards the influence of drying. For this purpose we gathered bloody gauze from operating rooms, blood-stained dressings, clothing from medico-legal cases, blood-soaked newspapers, and an old sponge soaked with rather putrid blood. Solutions made from materials of this nature being obviously of uncertain strength, we adopted the plan, also, of drying accurately measured amounts (.1 cc.) of serum, and of defibrinated blood (containing corpuscles) on clean glass slides. Such specimens were made twice weekly from the blood sent to the laboratory for Was-

sermann tests, thus affording means of comparing solutions of known strength of varying ages, wherein obviously the serum would have been subject to no deteriorating influence other than that of drying.

Table 3 shows results of such tests with dried specimens from one to six months old, with a serum of low potency. The results there indicated show plainly that drying deprives the blood of some of its power to react with the precipitin. The question immediately arises, would this hold good if a stronger anti-serum were employed? Applying our strongest serum (1-5000) to two specimens of dried serum, five and eight months old, respectively, we find (as shown—see Table 4) practically the same result. The

TABLE 3.

TEST JAN. 26, 1916.

A.: H. Serum, No. 8, Second Series.

No.	TITER	1/100	1/500	1/1000
	Fresh serum	+++	++	—
	Dried serum			
1	1/3/16	+++	++	$\pm$
2	12/3/15	+++	+++	++
3	11/2/15	+++	+	$\pm$
4	10/1/15	++	+	—
5	9/1/15	+	—	—
6	8/3/15	—	—	—
7	7/3/15	—	—	—
8	6/4/15	—	—	—

TABLE 4.

JAN. 28, 1916.

A.: H. Serum, No. 9, Second Series.

20 MINUTES	1/100	1/500	1/1000	1/3000
	Fresh serum		+++	++
	Dried serum			
9/9/15	+	—		
5/6/15	$\pm$	—		

solutions of the dried bloods were carefully made, and save for flocculi in some, which had to be filtered out, were complete, showing that lack of solubility was not the cause of failure to induce a reaction. Comparing No. 2, Table 3, with the fresh serum above, we find a more active reaction in the former, which represents serum dried nearly two months. The titer of the anti-serum there used was by previous tests established at 1-1000 (+), and the seeming anomaly suggests an *individual variation* in the power of blood to react with their homologous anti-sera.

Working with the specimen dried on various fabrics by extracting the stain with saline (.85) over night, centrifugating to greatest possible clearness and diluting to estimated strength of 1-200, except as otherwise indicated, and testing with both high and low value serum, 1-1000 and 1-5000, respectively, we obtain results shown in Table 5, which indicate again in general that the older the stain the less likely it is to react. The greater potency of anti-serum compensates somewhat for the inhibiting influence of drying,

TABLE 5.

TEST JAN. 18 AND 22, 1916.

A.: II. Serum R No. 8: 1/1000 value.

A.: II. Serum R No. 9: 1/5000 value.

NO.	TEST OBJECT	SOL.	REACT. LITMUS	COLOR	SERUM 1/1000	SERUM 1/5000
	Beef serum, fresh	1/100	Neut.	Pale	±	
	.85 saline solution		Neut.	Clear	—	
	RECENT					
1	Menstrual stain on trousers	1/500	Neut.	Pale straw	+++	
2	Ascitic fluid	1/17/16	Neut.	Very pale straw	+++	+++
	1 Gauze	1/200	Neut.	Pale straw	+++	+++
3	Pus dressing	1/17/16				
4	Blood on newspaper.....	11/17/15	Neut.	Pale brownish	+++	
5	Blood on newspaper.....	11/5/15	Neut.	Pale brownish	—	
6	Blood, abdominal dressing	9/14 (a)				
		1/200	Neut.	Reddish	±	+++
7	Blood, abdominal dressing	9/14 (b)				
		1/200	Neut.	Reddish brown	—	
8	Blood, sponge, foul.....	5/19/16	f - ac.	Reddish	+	+++
9	Blood, cloth	5/19/16	Neut.	Pale straw	+	+++
10	Blood, "Burke" Gauze ..	5/15/15	Neut.	Pale straw	—	—
11	Blood, shirt	18 mo. old	Neut.	Limpid	—	
12	Blood, victim's shirt	12/21/14	f-alk.	Reddish	—	—

and also seems to indicate, as above suggested, that there is variation in power to react with the precipitin in the blood of individuals of the same species, especially shown by Exp. No. 10 in Table 5, in which the test object was a gauze pad from the operating room, saturated with blood, put into a clean envelope and kept in the laboratory, in a drawer, and which could not have been contaminated, yet which has invariably resisted the precipitin test with several different sera, and is comparable to the results with the blood-stained shirt from the murdered man, which tested three months after the murder by one of the best serums I have produced, by the serum imported from Germany, and recently by our best serum (13 months after the murder) as shown in Table 5 has been invariably negative. To my mind these results suggest that there are certain bloods which react to a precipitin developed by serum from certain other individuals of the same species so weakly that drying completely inhibits them. An important deduction from the observations is that in an important case it would be well to save some of the fluid blood at the autopsy, which might be used to immunize, on the expectation of a more valuable serum for that individual, and to saturate some filter paper or gauze, and dry, to preserve for use as control in making the tests,—a precaution advised by Uhlenhuth, and called to the attention of this Society by Dr. Whitney fourteen years ago.

Recalling the case narrated at the outset,—our failure to reach a conclusion as to the identity of the stains found in the stable or on the murdered man's shirt by means of the precipitin test is noteworthy. On that account we are led to diverge from the specific subject of this communication briefly to cite the further pursuit of that important question,—were the stains made by human blood?

After the trial there remained to us the blood-

stained plank, the shirt mentioned, and a small bunch of hay chaff, matted together by a brownish substance which gave the guaiac and benzidin tests for blood, and was similar to the material which Professors Whitney and Balch had testified was mammalian blood.

Applying the complement-fixation method of Neisser and Sachs, we obtained a strongly positive test with the solution from the straw, and an absolutely negative one from an infusion of the unstained straw. This corresponded to the reaction by the same method from solutions made from the stains on the shirt. Solutions from the plank stains also gave positive reactions, but solutions from this source proved in control highly anti-complementary, and therefore useless for this test. The solution from the shirt was also found to be slightly anti-complementary, but we thought not sufficiently to discount the reaction completely. The solution from the hay stain was diluted to the point where anti-complementary power disappeared, and then showed a distinct binding of complement,—a reaction equivalent to the "1+" Wassermann test. Controls with beef and pig blood were negative. Solutions from the stain on gauze pad No. 10 in Table 5, and from the sponge with old putrid blood, both gave strong positive tests.

The authors Neisser and Sachs claim for their method a delicacy forty times that of the precipitin test, while Muir and Martin think it between 10 and 100 times more delicate. It certainly is more striking, and is not vitiated by slight cloudiness of the test solution. It is far more complicated and requires elaborate controls, and the outfit of a Wassermann laboratory is needed. Uhlenhuth claims that in the absence of a positive precipitin reaction, this test is insufficient to establish the source of the blood.

CONCLUSIONS.

From the results herein described we may draw the conclusions:

1. That the precipitin test *when it reacts* is a valuable and positive method of identifying blood stains (albumins). A negative test does not of itself *disprove* the presence of the homologous blood.

2. That the preparation of a serviceable serum may be a matter of weeks, and consequently a supply of adequate sera should be on hand, in order that the stains may not be subjected to prolonged drying before testing.

3. That in a murder case it would be of advantage to have blood from the victim in sufficient quantity to carry out an immunization, thus being better prepared to obtain a serum of high specific value.

4. That there may be individual variation in power of blood to respond to the precipitin test; consequently, failure to identify should not be considered final until other and stronger sera and the complement-fixation test have been tried.

Finally, we are glad to have occasion to pay a tribute of respect and admiration for the monumental labors of Professors Uhlenhuth and Nuttall and their co-workers and for the wonderful care, patient persistence and masterly insight with which they have, over a period of several years, studied by countless experiments the subject of precipitins, both in relation to their value to forensic medicine and in their bearing on biological problems, and by means of which they contributed appreciably to our knowledge of one of the processes by which Nature works her marvels of immunity, as well as finding important evidence of the blood relationship of certain species, supporting by tangible evidence certain conceptions of the evolutionists.

Our thanks are due to Professor William F. Whiting for the loan of Prof. Uhlenhuth's monograph, to our associates at Worcester City Hospital for saving and forwarding materials for the work, and to Dr. Frederick H. Baker for privileges in connection with the case cited.

[From the Laboratory of Pathology at Worcester Hospital.]

NOTES.

NUTTALL, p. 75 "In medico-legal work it will be desirable progressively to dilute a suspected blood sample, and to reach a conclusion upon the highest dilution (within limits) which reacts to a given anti-serum. In routine work, as I have stated, I have worked with dilutions of usually 1 : 100 to 1 : 200. As the dilution increases, the reaction narrows down more and more, the reactions with the highest dilutions being practically specific."

NUTTALL, p. 398. Ohamoto (4-1902) testing various human blood stains obtained negative results in about 1-7 of them. He found very old and putrid blood generally to give a negative result.

GRAHAM-SMITH, dilutions 1-21, using distilled water as the first solvent, made a series of tests on blood-stained implements from Scotland Yard. All

but one of 17 specimens, aged from 5 months to 30 years, were positive in from 5 m. to 1 hr., incubated at 37°C., except one which was mixed with rust and oil. Blood-stained fabrics from same source, 3 to 28 years old, obtained clouds in from 15 minutes to 1 hour in 10 out of 12.

UHELNHUTH tested upward of 60 specimens of dried blood from various animals as well as human sources placed at his disposal by the Prussian Minister of Justice, not being informed of the sources, with correct results in every case. He also records tests of mummified (preserved) specimens many years old, the source of which he was able to identify. Other stains he identified, varying from 6 to 12 years of age.

Many recorded results of tests, which state no definite values of sera used, are useless to us for purposes of comparison.

Both Nuttall and Uhlenhuth warn against the use of sera of high potency with low blood dilutions, on account of group reactions.

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FIBROMA OF MEDIASTINUM: REPORT OF A CASE.

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AMONG the diseases of the mediastinum, tumors, both benign and malignant, occupy a most interesting and important place. The differentiation of malignancy or benignity of such a tumor is more histological than clinical, since the ill effects are mostly due to pressure upon various vital structures situated in this limited area. A simple benign tumor, elsewhere sit-