

PATHOLOGIC EFFECTS OF SURAMIN, HETRAZAN AND ARSENAMIDE ON ADULT *ONCHOCERCA VOLVULUS**

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Great strides have been made within recent years in the treatment of filariasis. Onchocerciasis is the only filarid infection of man in which adult worms may be consistently obtained by biopsy. In view of this, the experimental therapy of onchocerciasis is of great value because the effects of drugs on adults may be determined. In other human filarid infections the effects of antimonials (1, 2) and hetrazan (3) on adult worms have been discussed on the basis of indirect evidence.

The effect of drugs on adult filarids is of importance in determining whether or not a drug is permanently effective in eliminating microfilariae. If the compound is effective only against microfilariae, then their recurrence may be expected.

Evidence has accumulated indicating that certain antimony compounds not only are microfilaricidal but have a sterilizing effect on adult *Dirofilaria* and *Onchocerca*. Hetrazan is microfilaricidal in *Wuchereria* (3), *Onchocerca* (4, 5) and *Dirofilaria* (6) infections. The arsenical preparation, arsenamide, is microfilaricidal in *Wuchereria* infections (7) and is effective against the adult *Dirofilaria* (8).

Suramin (Bayer 205) has been used successfully both against the microfilariae and adult *Onchocerca*. This was reported in a preliminary note by van Hoof, Henrard, Peel and Wanson (9). Burch (5) has treated 56 patients with this drug and found it to be very effective in causing disappearance of microfilariae.

The present study was designed to determine histologically the effects of suramin and hetrazan on the adults of *Onchocerca volvulus*.

EXPERIMENTAL PROCEDURE

Nodules containing adult *Onchocerca* were removed from patients during the study previously reported by Burch. In addition, 11 nodules removed by Dr. Luis Mazzotti from 3 patients treated with arsenamide and sent to us by Dr. Gilbert Otto are included in this study.

In this analysis, only those nodules are included which were removed from patients considered to have been adequately treated. Adequate treatment** in the case of suramin is considered to be a minimum total

* This work was done as a collaborative project of the National Institutes of Health and the Pan American Sanitary Bureau.

** Adequate treatment as defined above is not intended as a recommendation as to proper dosage of suramin and hetrazan in the treatment of onchocerciasis. The minimum dose indicated as adequate is only for the purpose of this analysis. In the case of hetrazan, the figure given may be too low.

dose of 0.14 gm./kg. and similarly with hetrazan a minimum total dose of 20 mg./kg. The 3 patients treated with arsenamide received this drug in 3 cc. doses (2% solution) daily for 15 days.

Using these criteria of adequacy of treatment, microscopic examination was made of 34 nodules from 21 patients receiving suramin and of 16 nodules from 9 patients treated with hetrazan. Nineteen nodules from untreated patients were used as controls for the study.

Nodules were fixed in 10% formalin and later blocked in such a manner that sections were made through what appeared the greatest concentration of adult worms. The tissue was embedded in paraffin, sectioned, and stained by azure eosin and by the Van Gieson or Masson techniques. Frozen sections of some nodules were stained with oil red O for the demonstration of fat.

RESULTS

Histopathology of Control Nodules.—The *Onchocerca* nodules were basically connective tissue masses, largely collagenous, infiltrated by variable numbers of inflammatory cells and showed from few to moderate numbers of fixed tissue cells. Throughout the masses sections of adult worms occurred, either widely separated or compactly grouped, depending on the number of worms present and the size of the nodule.

The fibrous tissue was usually quite dense at the periphery where it formed a fairly well delineated layer of variable thickness which is commonly referred to as a capsule. Some nodules (5 of 19) were densely fibrous throughout and in such cases there was no part that could be correctly considered as a capsule. Fibrous tissue in the central portion of the nodules usually was of only slight to moderate density and was roughly inversely correlated with the degree of inflammatory infiltration.

Fibroblasts generally were present in small numbers. In only one of the nodules was fibroblast proliferation particularly prominent. Large oval to round mononuclear cells with solid or foamy cytoplasm were seen in most nodules. When present in moderate numbers, they tended to be concentrated in patches in the stroma or about the worms. Fat stains done on a few nodules showed much neutral fat both in the large mononuclears and in the spindle shaped stroma cells. In an occasional nodule a sheath of irregularly oriented oval to spindle-shaped histiocytes occurred about one or very few worm segments. This sheathing was seen only around worms showing some degenerative change. A single clear cut granuloma of foreign body type was found. An occasional giant cell of the foreign body type was seen in a little more than half of the nodules.

Cellular infiltration of different nodules varied markedly both as to degree and cell type. In two, the tissue was densely infiltrated. It was of moderate degree in 5, slight in 8 and very slight in 4. In those cases

showing only slight infiltration, the cells usually occurred in foci or clustered about the adult worms. The infiltrating cells were lymphocytes, plasma cells, eosinophils and polymorphonuclear leucocytes. Taking the nodules as a group, no one of these cell types could be considered as predominant but in different nodules and in different areas of the same nodule, any one of them may form the bulk of the infiltrate. Of the lymphoid cells, lymphocytes were seen in a greater number of nodules, but in some foci plasma cells were abundant. Eosinophils were the most variable cell as to frequency of appearance. They were not seen in five nodules but were numerous in two. They usually showed a patchy distribution but there was no tendency for them to be concentrated in the vicinity of adult worms or about microfilaria present in the stroma. Polymorphonuclear leucocytes generally were present in quite small numbers and were most often seen in areas of tissue softening or necrosis around segments of worms. Such areas of necrosis, usually quite small, were seen in all nodules. Medium sized areas of necrosis, tissue collapse, fluid and polymorphonuclear leucocytic exudation were seen in 6 of the 19 nodules.

Histology of Adult Onchocerca in Nodules from Treated and Untreated Patients.—Because of the entwined position of female *Onchocerca* in the nodules, a single section usually passed through various levels of the worms. By study of multiple nodules from untreated patients, it was easily possible to follow the reproductive sequence from ovary to the fully developed microfilariae. It appears that morphologically the sequence of differentiation is similar to that described and illustrated for *Dirofilaria immitis* (10). In view of the fact that the ovary is restricted to a relatively short segment of the worm, it was seen infrequently.

The majority of female *Onchocerca* in the 19 control nodules from untreated patients showed the normal developmental stages and numerous microfilariae in their uteri. Occasionally one or two ova (in uterus) or multicell stages showed karyorrhexis or frank necrosis. There were a few worms which showed definite abnormalities. These changes varied from necrosis of uterine contents of variable extent to death of the worm.

In an effort to grade the worms of both control and "treated nodules" and to arrange them in order from normal to severe damage, the females of each nodule were classified as follows:

Group I. The worms placed in this category showed developmental forms in the proper uterine level and morphologically differentiated microfilariae in the anterior portion of the uteri. Some worms showed slight variation from normal in the tinctorial quality of the cytoplasm of the cells of the developing and differentiated microfilariae. Since the significance of this minor change was not clear, these worms were considered essentially normal and placed in this group.

Group II. The worms placed in this group usually showed both developmental stages and differentiated microfilariae. However, when present they

showed frank necrosis which in many instances was extensive. Worms which did not show microfilariae but in which a few multicell forms were present, were also included in this group.

Group III. In this group were placed those worm which showed complete suppression of microfilarial production and absence of developing stages. The uteri of the worms of this group, at most, contained a few undeveloped and often degenerating ova.

Group IV. This group includes dead worms and a few nodules showing clear-cut foreign body granulomas which beyond doubt marked the site of recently disappeared worms.

The classification given above is similar to that used in a previous report (11) in which the effect of treatment with trivalent antimony was evaluated. The only significant difference is that dead *Onchocerca* and nodules showing only foreign body granulomas are placed in group 4 whereas they were previously included in group 3. Of all groups, group 2 contains worms which showed most variation in severity of damage. This may possibly mask minor differences of therapeutic effectiveness between hetrazan and suramin. However, in view of the number of variables in the study, such as dose of drug and interval between completion of treatment and removal of nodules, subdivision of this group was not practical.

For reasons given earlier, ovaries were seen in a relatively small number of worms. In these no clear-cut changes were found. Male worms also were recognized infrequently.

Using the criteria outlined above for separating the worms (nodules) into four groups a comparison of findings in treated and untreated patients is made in Tables 1 and 2.

Treatment with Suramin.—The data in Table 1 show that adequate treatment with suramin has an early marked suppressive effect on microfilarial production and ultimately causes death of the adult *Onchocerca*. In the specimens removed 60 days or less following completion of treat-

TABLE 1.—Comparison of histopathologic findings (groups*) in female *Onchocerca* in untreated patients and patients adequately treated with suramin (Bayer 205)

Group*	Number of Nodules			
	Untreated Controls	Treated		
		Post-treatment interval—days		
		60 or less	90-120	121-323
1	13	0	0	0
2	2	14	0	0
3	3	1	6	1
4	1	2	3	7

* See text for histologic basis of classification by groups.

TABLE 2.—Comparison of histopathologic findings (group*) in female *Onchocerca* in untreated patients and patients "adequately" treated with hetrazan

Groups*	Number of Nodules		
	Untreated Controls	Treated	
		Post-treatment interval—days	
		60 or less	61-90
1	13	8	2
2	2	1	2
3	3	0	3
4	1	0	0

* Same as table 1.

ment, there were no normal worms. The majority (group 2) showed either necrosis of variable extent of the uterine contents or uteri containing only normal or degenerating ova. In 90 to 120 days the changes were more striking. The worms of 3 nodules were dead (group 4) and 6 showed complete suppression of reproduction (group 3). After an interval of 121 to 323 days, 7 of 8 nodules showed only dead worms or foreign body granulomas. Photomicrographs illustrating normal *Onchocerca* and changes that occurred following treatment are shown in Figures 1 to 7.

Treatment with Hetrazan.—Table 2 shows the grouping of female *Onchocerca* from nodules after "adequate" treatment with hetrazan. Nodules removed within 60 days of completion of treatment showed no effect either on microfilarial production or on the adult worms. The worms of 8 of 9 nodules were normal. After 61 to 90 days some effect of treatment became evident. The worms of 2 nodules were normal, 2 showed minimal degenerative changes in uterine contents and 3 showed complete suppression of reproduction.

Treatment with Arsenamide.—The 11 nodules from the 3 patients treated with arsenamide showed only one with normal adult worms. In 9, the worms showed complete suppression of reproduction (group 3) and in one, the *Onchocerca* uteri contained a few ova but no multicell stages or microfilariae. None of the worms appeared dead.

Histological Appearance of Nodules Following Treatment.—The changes that occurred in nodules following treatment appeared to be related to pathologic alteration of the *Onchocerca* and not to the specific drug. Hence nodules from all patients treated with the three drugs employed are considered together in this section. As stated earlier in this report, nodules from untreated patients show considerable variation as to degree and type of cellular infiltration, necrosis, and amount of fibrous tissue. In view of this fact, only very general statements can be made as to the influence of treatment on these features of the nodules. Aver-

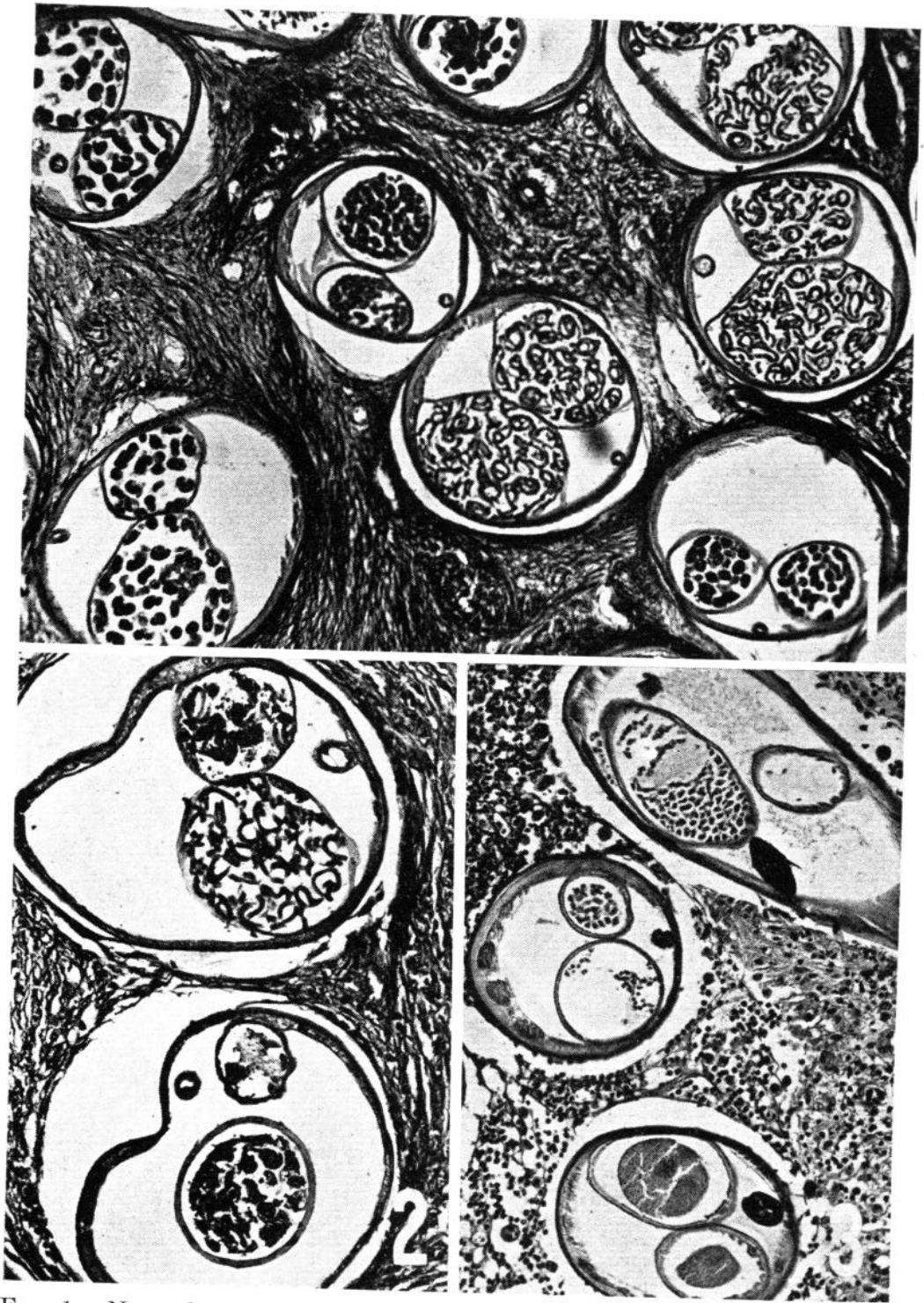


FIG. 1.—Normal *Onchocerca volvulus* showing active reproduction. Well differentiated microfilariae are seen in a number of cross sections. The most immature stages in this photomicrograph are at lower right. Tadpole stages are seen at upper and lower left. Van Gieson stain. $\times 125$. Photomicrographs 2 to 7 are of nodules removed from patients following treatment with suramin.

FIG. 2.—Essentially normal microfilaria are seen in one cross section of uterus; others show karyorrhectic necrosis of developing (multicell) stages. This worm was classified as group 2. Hematoxylin azure eosin stain. $\times 125$.

FIG. 3.—Shows only early developing stages, many of which are necrotic. Microfilariae were not seen in the uteri of this worm (group 2). Hematoxylin azure eosin stain. $\times 130$.

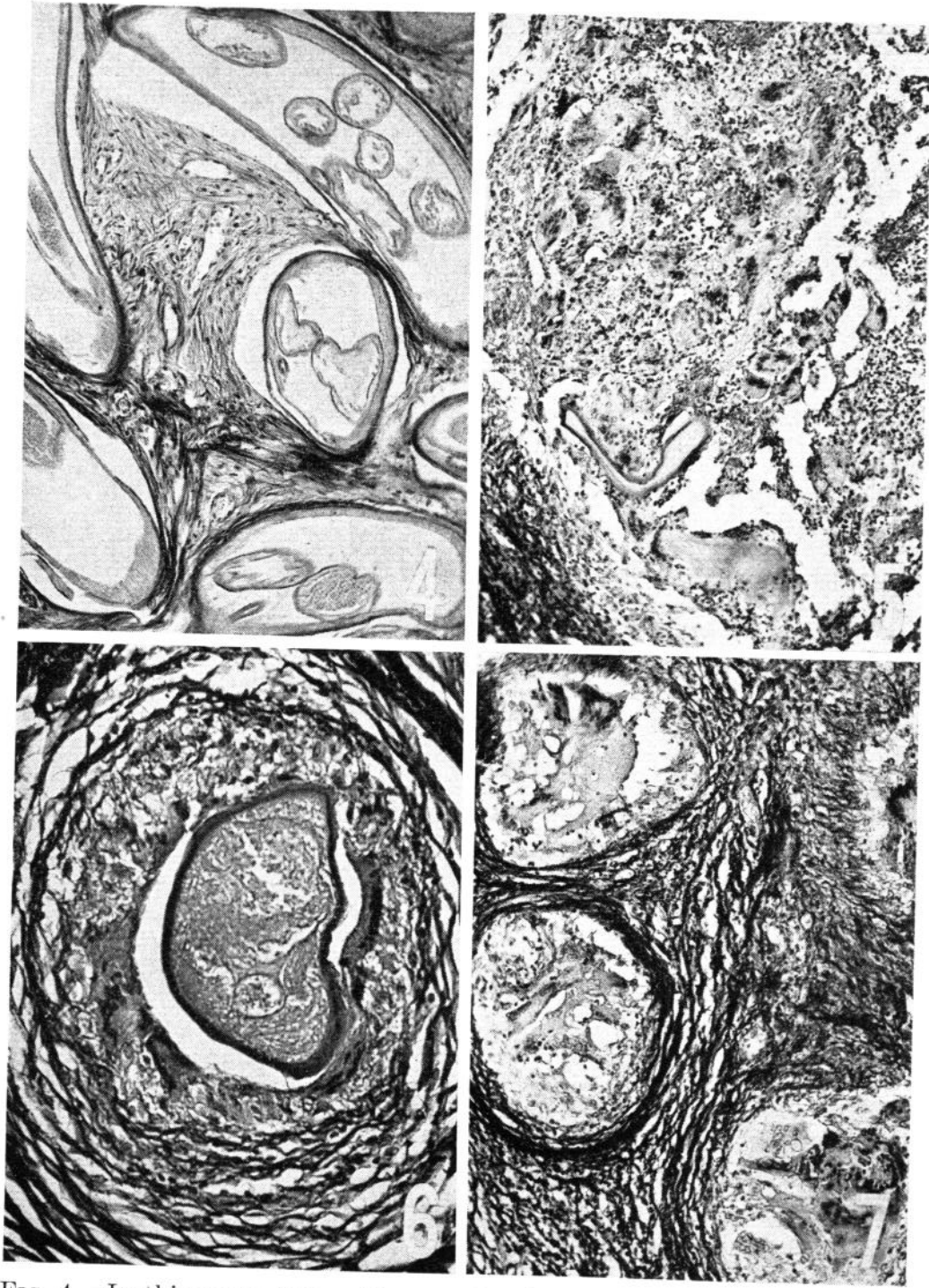


FIG. 4.—In this worm (group 3), reproduction is markedly suppressed. Uteri are empty or contain only a few non-differentiating ova. Van Gieson stain. $\times 100$.

FIG. 5.—Shows necrosis, polymorphonuclear leucocyte infiltration, and marked giant cell reaction to fragments of collapsed and dead *Onchocerca* appearing near bottom. Van Gieson stain. $\times 125$.

FIG. 6.—Dead worm surrounded by a prominent histiocyte and giant cell sheath. Van Gieson stain. $\times 220$.

FIG. 7.—Foreign body granulomas marking site of resorbed worms. Van Gieson stain. $\times 125$.

age total cellular infiltration was not apparently altered by treatment but in many nodules of group 4, especially those showing few or no worms, there were relatively fewer inflammatory cells present, and among these, lymphocytes predominated. Eosinophils were seen less frequently. In nodules of group 2, which includes worms showing acute degenerative changes, large mononuclear cells appeared to be increased in number. Areas of necrosis, usually with associated polymorphonuclear leucocytic exudation, showed the same variation in size noted in controls and showed no definite increased frequency that could be appreciated by histologic examination. In nodules of group 4, fibrous tissue was definitely increased in the main central portion and blended with the commonly densely fibrous "capsule."

Three features of certain nodules from treated patients contrasted sharply with nodules from untreated patients. These were sheathing of worms by oval or spindle-shaped histiocytes, frequency of giant cells of foreign body type and the presence of few to many foreign body granulomas. The histiocyte sheaths were of variable thickness and stood out in sharp contrast against the surrounding fibrous tissue in those nodules showing relatively little cellular infiltration. Multinucleate giant cells, often very large and of irregular shape, were numerous in many nodules. They generally were seen adjacent to worms intermingled with and forming part of the sheaths mentioned. They also were seen as part of foreign body granulomas, some of which were formed largely of these cells. These granulomas were seen in a few nodules of group 2, in a greater number of nodules of group 3, and were present in practically all nodules of group 4. Giant cells and histiocyte sheathing also increased in frequency and prominence in nodules classified from group 2 to 4.

Microfilariae are found in the stroma of most nodules removed from untreated patients and appear to be related to the reproductive activity of the adult. In this connection the following data from all treated (whether adequate or not) patients is cited. Microfilariae were not found in any nodules classified in groups 3 and 4. They were present in all 16 classified as group 1 and in approximately half of the 29 classified as group 2.

DISCUSSION

The goal of treatment of onchocerciasis is the killing of the disseminated microfilariae as well as the adult worms in the nodules. Van Hoof *et al.* (9) in a preliminary note reported that Bayer 205 (suramin) caused the disappearance of microfilariae from the tissues and that dead and flabby adults were found in the nodules. Burch (5) in a series of 56 cases also found the suramin to be an effective microfilaricide and the present report, which is a part of the same study, gives histopathologic evidence as to the drug's lethal effect on the adult worms. Definite injury of the adults can be seen in those nodules removed within 60 days following

completion of treatment. However, incontrovertible evidence of the drug's lethal effect was obtained only from those nodules removed after a 121 to 323 day interval; most were nearer the latter period. Good evidence of the drug's potential lethal effect was seen in nodules removed 114 days following completion of treatment. The worms of many such nodules showed changes that were considered to be irreversible. Distortion, collapse, and fragmentation were not infrequent and occasionally portions of worms were dead. The histocyte sheathing and giant cell reaction is interpreted as indirect evidence of damage to the adult *Onchocerca*.

This histologic study does not permit a definite conclusion as to the effect of hetrazan on the adult worms. This is true since the number of "adequately" treated cases is small and since all nodules were removed 90 days or less following completion of therapy. The 7 nodules studied after a 61 to 90 day interval indicate a suppressive action on microfilarial production but no changes were seen in the adults which would suggest that death was likely to occur. Examination of nodules after a longer post treatment period will be necessary before a definite conclusion can be drawn as to the effect of hetrazan. However, suramin produced a definite suppression of reproduction within 60 days after treatment whereas a similar but less pronounced effect was evident with hetrazan only after a longer period (61-90 days).

The patients treated with arsenamide are too few to permit any conclusion. However, the changes seen in the worms from these cases were such as to suggest that the drug is worth further trial.

SUMMARY AND CONCLUSIONS

Histopathologic examination was made of 34 onchocercotic nodules from 21 patients who had been treated with a minimum of 0.14 gm./kg. (total dose) of suramin, of 16 nodules from 9 cases treated with a minimum of 20 mg./kg. (total dose) of hetrazan and of 11 nodules from 3 cases treated with 3 cc. of arsenamide daily for 15 days. The nodules were removed after intervals of 29 to 323 days following completion of treatment. From this study it was evident that suramin had a lethal effect on adult *Onchocerca* which was demonstrable in nodules removed after 121 days. In most nodules removed after shorter intervals, there usually were considerable pathologic alterations of the uterine contents.

A definite conclusion as to the effect of hetrazan on adult worms cannot be made from this study. This drug caused some suppression of reproduction but its action in this respect was slower than that of suramin.

The changes seen in the worms of the 3 patients treated with arsenamide was of such nature and degree as to suggest that further experimental trial of this drug is indicated.

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EFFECTOS PATOLÓGICOS DE LA SURAMINA, EL HETRAZÁN Y LA ARSENAMIDA SOBRE LA *ONCHOCERCA VOLVULUS* ADULTA (*Sumario*)

En el tratamiento de las infecciones filáricas es importante conocer el efecto de la droga sobre los vermes adultos. Para ser completamente efectiva ésta, debe exterminar los vermes adultos o esterilizar permanentemente a las hembras. La oncocerciasis es la única filariasis del hombre en la cual pueden obtenerse consistentemente vermes adultos por medio de la biopsia.

Este estudio se realizó con el fin de determinar histológicamente el efecto de la suramina (Bayer 205) y del hetrazán sobre los vermes adultos de *Onchocerca volvulus*. En un estudio previamente publicado por Burch (véase *Boletín*, mzo. 1949) se extirparon nódulos conteniendo vermes adultos, en enfermos voluntarios residentes en la zona endémica de Guatemala. Los nódulos fueron extirpados de 29 a 323 días después de completar el tratamiento. En este análisis se tomaron en cuenta solamente aquellos enfermos que habían recibido un mínimo de 0.14 gm/kg (dosis total) de suramina, o 20 mg/kg (dosis total) de hetrazán. Por cortesía de los Dres. Mazzotti y Otto, se incluyeron 3 casos tratados diariamente con 3 cc. de una solución de arsenamida al 2% durante 15 días. Sirvieron como testigos diecinueve nódulos de enfermos no tratados.

Las alteraciones patológicas inducidas por la suramina variaron desde necrosis de algunas formas intrauterinas en desarrollo y microfilarias hasta la muerte y reabsorción completa de los vermes adultos. En algunos vermes se hallaba vacío el útero o contenía sólo pocos huevos frecuentemente en estado de degeneración. En los nódulos eliminados 60 días o menos, después de completar el tratamiento, no existían vermes normales; en dos de catorce nódulos los vermes estaban muertos. Después de 90 a 120 días, tres estaban muertos y seis mostraban marcada supresión de la reproducción. De ocho, siete estaban muertos después de los 121 a 323 días. Es indudable que la suramina posee efecto rápido y marcadamente supresivo sobre la producción microfilarica y finalmente produce la muerte de la *Onchocerca* adulta.

Este estudio no permite llegar a una conclusión definitiva en cuanto al efecto del hetrazán sobre los vermes adultos, lo que se debe al escaso número de casos y el intervalo relativamente corto entre la terminación del tratamiento y la remoción de los nódulos. No apareció lesión evidente en el término de 60 días o menos. Sin embargo, el examen de los 7 nódulos removidos después de 61 a 90 días de haber terminado el tratamiento, indicó acción supresiva en la producción de microfilarias. No se observaron vermes muertos en ninguno de esos nódulos.

Las alteraciones observadas en los vermes de los tres casos tratados con arsenamida fueron de tal grado y naturaleza, que justifica nueva terapia experimental con dicha droga.

Se relacionaron las alteraciones definidas en la estroma de los nódulos observadas después del tratamiento, con los vermes patológicamente alterados. Estas alteraciones consistían del recubrimiento de los vermes por histiocitos, aumento en el número y tamaño de las células gigantes, formación de granulomas de tipo de cuerpo extraño marcadamente delineados, y fibrosis.

DESCRIPCIÓN DE LOS GRABADOS

(Véase pp. 1112 y 1113)

Fig. 1.—*Onchocerca volvulus* en reproducción activa. Obsérvanse microfilarias bien diferenciadas en algunas secciones transversales. Los estados más inmaturos aparecen en la parte inferior derecha de la microfotografía; los estados de renacuajo, en la parte izquierda, superior e inferior. Coloración Van Gieson. $\times 125$.

Las microfotografías 2 a 7 representan nódulos removidos después del tratamiento con suramina.

Fig. 2.—Microfilarias esencialmente normales en una sección transversal del útero; otras muestran necrosis cariorrectal del desarrollo (multicélulas). Este verme fué clasificado en el grupo 2. Coloración hematoxilina-eosina azul. $\times 125$.

Fig. 3.—Estados tempranos de desarrollo, muchos de ellos necróticos. No se observan microfilarias en el útero de este verme (grupo 2). Coloración hematoxilina-eosina azul. $\times 130$.

Fig. 4.—En este verme (grupo 3), la reproducción se halla marcadamente reprimida. Los úteros se hallan vacíos o contienen solamente unos pocos huevos no diferenciados. Coloración Van Gieson. $\times 100$.

Fig. 5.—Muestra necrosis, infiltración de leucocitos polimorfonucleares y marcada reacción de células gigantes a fragmentos de *Onchocerca* en colapso y muertas (parte inferior). Coloración Van Gieson. $\times 125$.

Fig. 6.—Verme muerto rodeado de un histiocito prominente, cubierto de células gigantes. Coloración Van Gieson. $\times 220$.

Fig. 7.—Granuloma de cuerpo extraño, que marca el sitio de los vermes reabsorbidos. Coloración Van Gieson. $\times 125$.