

hydriyl mechanism is involved in one or all of these reactions.<sup>7</sup> Further study of various chemotherapeutic agents and heavy metal compounds is in progress.

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Mechanism of Chemotherapeutic Action. II. Rôle of Reticulo-endothelial System in Formation of a Parasitotropic Agent from Arsenicals.

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Recently many investigators<sup>1-10</sup> have attempted to study the rôle of the host in chemotherapeutic action by investigating the influence of blockade of the reticulo-endothelial system. They found that different chemotherapeutic agents were less effective with blocked, or blocked and splenectomised animals than with normals. From prior experiments<sup>11</sup> it appears that even as active an agent against trypanosomes as Bayer 205 needs the host's normal protective mechanism for the destruction of the parasites. Thus it was to be expected that blockade would influence unfavorably the course of an infection whether or not a chemotherapeutic agent was also applied. Confirming this view, Kikuth and Regendanz's experiments indicate that treated and non-treated infections are influenced similarly by the blockade. On the other hand, it is known<sup>12</sup> that arsenicals, especially the less diffusible, trivalent compounds

<sup>7</sup> Voegtlin, Dyer and Leonard, *U. S. Public Health Rep.*, 1923, xxxviii, 1882; *J. Pharmacol. Exp. Therap.*, 1925, xxv, 297.

<sup>1</sup> Kritschewsky, I. L., and Meersohn, J. S., *Z. Immunitäts.*, 1926, xlvii, 407.

<sup>2</sup> Kritschewsky, I. L., *Z. Immunitäts.*, 1927, liii, 506; *Centr. Bakt. Parasitenk. Orig.*, 1927, civ, 214; *Z. Immunitäts.*, 1928, lix, 1.

<sup>3</sup> Kritschewsky, I. L., Baskin, M. M., and Lebedjeva, M. N., *Centr. Bakt. Parasitenk. Orig.*, 1930.

<sup>4</sup> Kolpikow, N. W., *Z. Immunitäts.*, 1926, xlviii, 182.

<sup>5</sup> Jungeblut, Cl. W., *Z. Hyg. Infektionskrankh.*, 1927, cvii, 357.

<sup>6</sup> Jungeblut, Cl. W., and McGinn, Barbara B., *J. Exp. Med.*, 1930, li, 5.

<sup>7</sup> Feldt and Schott, *Z. Hyg. Infektionskrankh.*, 1927, cvii, 453.

<sup>8</sup> Jancso, N. v., *Z. ges. exp. Med.*, 1926, lxi, 63; 1929, lxxv, 98.

<sup>9</sup> Schlossberger, H., *Centr. Bakt. Parasitenk. Orig.*, 1929, cx, 210.

<sup>10</sup> Kikuth and Regendanz, *Z. Immunitäts.*, 1929, lxi, 422.

<sup>11</sup> Reiner and Köveskúti, J., *Deut. Med. Wochschr.*, 1927, liii, 1988.

<sup>12</sup> Voegtlin, C., and Thompson, J. W., *J. Pharmacol. Exp. Therap.*, 1922, xx, 85.

are bound and stored in liver and spleen. It was noted especially by Voegtlin,<sup>13</sup> Dyer and Miller that storage favors the chemotherapeutic action. Theories have also been advanced according to which the chemotherapeutic agent stimulates the reticulo-endothelial system<sup>7</sup> or this system inhibits the agent from exerting its action<sup>8</sup> or transforms it into a more active agent. There are no experimental proofs that the influence of the reticulo-endothel is more than what one could expect from its known functions in intermediary metabolism and in detoxification.

Since we<sup>14</sup> have demonstrated that arsenicals are transformed in the host into a more active parasitotropic agent, we thought to obtain some idea of the importance of the reticulo-endothel in chemotherapeutic action by investigating its influence on formation of this active agent in non-infected, arsenical-treated animals. Thus we eliminated the influence of the blockade on the infection and were permitted to study separately its possible influence upon the chemotherapeutic agent.

Our procedure was as follows: Animals (rats and rabbits) were injected intravenously with 5 cc. per kilogram of india ink, diluted 1:5 with physiological salt solution. Twenty hours later these animals and controls received equal amounts of arsenicals intravenously (sodium atoxylate was given intraperitoneally). Doses: 100 and 250 mg. per kilogram. One day later (in one experiment, 3 hours later) the animals were bled. The plasma of blocked and non-blocked animals was diluted with 3.5% sodium citrate in the same proportion (1:2 or 1:3). These plasma were tested for their activity by the combined *in vitro* and *in vivo* method, described previously,<sup>14</sup> using for each blood sample 2-3 rats. Virulence controls of the trypanosomes were treated similarly with normal plasma, likewise diluted in the same proportion with citrate. In cases where the effect was slight the test was later repeated with the same blood sample and always with exactly the same result, indicating a high accuracy and sensitivity of the method and that the virulence of the control trypanosomes was kept unchanged. The arsenic content of the blood-plasma, spleen and liver was estimated by the Gutzeit method. For relative estimation of neoarsphenamine, we also used the method suggested by Hiramatsu.<sup>15</sup> It was never found that the blocked animal's blood was less active than that of

<sup>13</sup> Voegtlin, C., Dyer, H., and Miller, J. *Pharmacol. Exp. Therap.*, 1922, **xx**, 129.

<sup>14</sup> Reiner, L., and Leonard, C. S., *Proc. Soc. Exp. Biol. and Med.*, 1930, **xxvii**, 788.

<sup>15</sup> Hiramatsu, *Sci-i-Kwai Med. J.*, **xlvi**, 133; *Eng. Abst.*, 1929, Sect. 5.

the non-blocked, which would follow if the reticulo-endothel tended to enhance chemotherapeutic activity. In 3 experiments out of 5 (twice with neoarsphenamine, once with arsphenamine) we found that the blood of blocked animals was more active than that of non-blocked. In 2 experiments the difference was small but constant on repetition. In another experiment in which 250 mg. per kilogram was injected, bleeding 3 hours later, animals lived twice as long as those infected with trypanosomes treated with non-blocked, arsenic plasma, although these also showed a marked protection. Arsenic content of plasma was in these cases higher in blocked animals than in non-blocked. The arsenic content of the liver did not differ in blocked and non-blocked animals, whereas the non-blocked spleen usually contained more arsenic than the blocked.

We conclude that "*blockade*" does not inhibit the formation of the parasitotropic agent in the host, but rather may enhance it. Since the blockade tends to increase the arsenic content of the blood of animals treated with arsenicals, it is probable that the increased activity is due to an increased concentration of the active principle. But the possibility should not be overlooked that other changes of the blood plasma produced by the blockade<sup>16</sup> may have influenced the results of our experiments. That the reticulo-endothelial system was "stimulated" is not probable, for we used large doses of india ink and found changes in the distribution of arsenic which must be attributed to a fairly high degree of blockade.

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### Relation of Temperature to Nutrition and Resistance.

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With our studies on the effect of external environment on susceptibility and resistance of animals to infection, we are also studying the indirect effect through the influence on the nutrition. We<sup>1</sup> found that rats fed high fat-containing diets (20-35% of the calory

<sup>16</sup> Wichels, P., *Z. ges. exp. Med.*, 1926, liii, 287.

<sup>1</sup> Geiger, A., Mueller, R., and Kligler, I. J., *Biochem. Z.*, in press.