THE MECHANISM OF THE INCREASE IN RESISTANCE AGAINST LETHAL DOSES OF HISTAMINE DEVELOPED BY PREVIOUS INJECTIONS OF SMALL HISTAMINE DOSES.

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(With 1 Figure and 4 Tables in the text.)

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In a previous paper (Beznák, Kovách, Gáspár-Rády, 1946), we have shown that repeated injections of small doses of histamine (HT = histamine treatment) cause in certain guinea pigs an increase of resistance against the normal 100% lethal dose. Such animals with increased resistance succumb only to a dose which is 3—6 standard deviation far from the normal ALD. As described in the paper cited, the HT does not increase the resistance of all animals of the treated population evenly, it leaves the resistance of many of them unaltered.

In the present paper we describe our experiments dealing with the physiological mechanism of the increase in this resistance.

Previous works: There are few papers describing direct experiments bearing on the physiological mechanism of H-resistance. It was claimed by Karády (1935) that the fall in the arterial blood pressure in H-desensitized cats is smaller than in normal ones. Kokas, Sarkady and Went (1938) could not confirm this result. Control experiments carried out by one of us in collaboration with Csáky (Csáky & Kovách, 1947) proved that neither of the two experimental procedures was satisfactory. Experiments in which the complete concentration-action curves were compared in the same cats before and after HT proved clearly that HT does not alter the arterial blood pressure response to H in the cat.

HT was found in rabbits to diminish the decrease of the blood volume after a large dose of H and after traumatic shock. (Karády, 1938.) This same was found earlier in dogs as far as the traumatic shock is concerned (Rusznyák, Karády & Szabó, 1936).

A more or less complete theory considers HT as a special case of the "alarm reaction of SELYE" (KARÁDY, SELYE & BROWNE, 1938 a, b). According to the alarm reaction theory, the first application of any non-specific, fairly strong stimulus (injection of formaldehyde, application of cold, physical exercise) provokes from the organism the first phase of the alarm reaction. By this the animal becomes more resistant to the second application of any such non-specific alarm stimulus. In the development of this resistance the hyperfunction of the suprarenal cortex plays a decisive role (SELYE, 1937). Independent of the question of whether HT is a special case of the alarm reaction postulated by SELYE's theory or not, it is a fact that it causes in the rat suprarenal hypertrophy (MACKAY and CLARK, 1938). The circumstance that non-specific stimuli of varied kinds may be neutralized by the same single agent (the suprarenals) is explained hypothetically by supposing that all of them release histamine, and it is this hypothetical common physiological denominator which the hyperfunctioning suprarenal counteracts. (SELYE, 1937). It is indeed well known that the H-resistance is decreased in the epinephrectomized animal and is restored by desoxycorticosteronacetate. (NOBLE & COLLIP, 1941). In the blood of adrenalectomized animals the H-content is 21—230% elevated and suprarenal cortex-extract restores it to almost the normal level. It is significant that in fake operations which may be regarded as an alarm stimulus, the H-content decreases (WILSON, 1941). The mode of action of the suprarenals, both in the alarm reaction and in the H-desensitization, is unknown.

A few words must be said about the mechanism of the acute increase of resistance to H caused by rapidly repeated injections of H (H-tachyphylaxis). It was proved by EICHLER & KILLIAN (1931) and by EICHLER & MÜGGE (1931) that in this resistance the developing incompensated acidaemia plays a role. Acidaemia relaxes the spasms of the bronchioles caused by H-injections. With increasing doses of H the acidaemia is also increased but not the broncho-constriction. It may be mentioned in this connection that M. BEZNÁK, KORÉNYI & HAJDU (1942) produced evidence that the suprarenal hypertrophy and hyperfunction caused by exercise is brought about by the increase of the concentration in the blood of muscle metabolites, primarily of acids.

In planning the course of our search for the mechanism of the increase of resistance, we proceeded as follows: Guinea pigs made resistant by HT are killed by a dose considerably higher than the 100% normal LD. The symptoms of the mechanism of death do not differ in the normal and in the resistant, HT guinea pigs. The mechanism of death caused by H, as proved by the experiments of DALE and LAIDLAW (1910—11), consists, in the guinea pig, of suffocation due to a diminished output of O_2 blood by the left ventricle. This is the consequence of a very powerful broncho-constriction and diminution of the blood-flow through the lungs. This latter in its turn is caused partly by the constriction of the pulmonary arterioles, partly by the failure of the heart ventricle which is conditioned by the inadequate supply of oxygenated blood. If therefore a 100% LD of H does not

kill a resistant HT animal, this must be due to either a better output of O_2 blood or to an increased resistance to lack of O_2 blood by the tissues. O_2 determinations carried out at the peak of H-shock caused by a 100% LD of H in the arterial blood of normal, non-resistant- and resistant- HT guinea pigs, decided in favour of the first possibility: the lungs of the resistant HT animals oxygenate far better than do the normal or the non-resistant guinea pigs.

Theoretically there are two possibilities which might bring about this change. HT either made the broncho- and vaso- constrictors in the lungs less responsive to the same level of H in the blood, or reduced the concentration of H in the blood following the s. c. injection of the same dose of H. Determinations of the H content of the blood showed a small and irregular tendency towards a reduced H content in the HT animals. These estimations showed, therefore, that the better oxygenation is the consequence of the decreased response of the broncho- and vaso-constrictors to the same blood level of H. Estimations of the rate of adsorption from the peritoneal cavity and estimations of the rate of disappearance of i. c. injected H have also been made, to find out whether this small and irregular decrease was not due to an alteration of the dynamic equilibrium between these two factors.

Since EICHLER *et al.* showed that incompensated acidaemia causes the bronchoconstrictors to relax, we carried out comparative lactic acid and CO_2 determinations in the blood.

METHODS.

In the comparison of the blood O_2 and CO_2 content, 37 male guinea pigs of 611 g $\mu \pm 34$ g were used. The experiment were made between 17. 7. 1944 and 31. 12. 1944. In 8 of them we determined the O_2 and CO_2 content of the normal carotid blood of the guinea pigs (Group. I.). All animals survived this operation. In 5 normal guinea pigs we suceeded in taking presumably premortal blood from the carotid at the peak of the H-poisoning (Group II.). A few minutes later all animals died. 24 animals had been pre-treated with H as described below. At the conclusion of the HT they were given the normal 100% LD (410 μg/100 g). When menacingly severe symptoms occurred blood was drawn from the carotid. The animals which died make up the non-resistant HT Group III.; those which survived form the resistant HT. Group IV; If such symptoms did not occur we waited

for 30 minutes, and carried out the O_2 and CO_2 determinations in the carotid blood. These animals are also registered in the resistant HT Group IV.

The withdrawal of blood was carried out as follows: The animal was tied to the operating table. In local novocaine anaesthesia the external jugular vein was cannulated, 3 mg/100 g of Liquoid La Roche was slowly injected to prevent clotting. Canulla was introduced into the carotid on the same side. The 100% s. c. normal lethal dose, 410 μg/100 g b. w. in 0.1 ml. of tyrode, was injected into the subcutis of the upper, middle abdominal region. The time of the withdrawal of blood is described above. Between 2—4 ml of blood were withdrawn. In 1 ml the O_2 and CO_2 contents were determined, by VAN SLYKE's manometric method (1927). In 1 ml. of blood the H content was determined by CODE's method (1937) as modified by EICHLER and SPEDA (1940).

The HT was carried out as follows: on the 1st and 2nd days 0.01, on the 3rd and 4th 0.02, from the 5th day onwards for 14 days 0.03 mg/100 g. b. w. H was given twice a day 0.1 ml/100 g. b. w. of tyrode s. c. into the upper middle abdominal region.

The rate of adsorption from the peritoneal cavity was determined in 12 normal and 12 desensitized animals. These were given 650 μg/100 g b. w. H base i. p. (60% of the 100% normal i. p. LD into the lower middle part of the abdominal region. Half an hour later the animals were decapitated and bled out, the abdomen laid open, its content allowed to drain thoroughly and the abdominal cavity washed 3 times with saline. These determinations were carried out between July 22nd, 1943 and September 12th of the same year. The H determinations were carried out with BARSOUM & GADDUM's method as modified by CODE (1937). The HT of these animals began with 10μg on the first and 2nd day, then the dose was raised as follows: 3rd and 4th days, 20; 5th and 6th, 30; 7th 40, 8th and 9th days 50, 10th day 60, 11th day 70, 12th 80, 13th 90, 14th 100 μg. Each dose was given once a day. Each dose in μ gs/100 g. b. w.

The determinations of lactic acid in the blood were carried out with the method of LAURENSEN and WAHLLÄNDER (1938).

In a few cases we determined the blood flow from the carotid using the following method: The guinea pig was given 3 mg/100 g. b. w. Liquoid La Roche in 0.2 ml. of tyrode solution in the external jugular vein. The left carotid was cannulated in Novocaine anaesthesia, tests of the blood flow were taken at intervals by releasing the Spencer-

Wells in the carotid and then counting with a stop-watch the time intervals of drops of blood.

EXPERIMENTAL RESULTS.

In Table I. we present the results of determinations of O_2 and CO_2 in the carotid blood.

TABLE I.

The O_2 and CO_2 content of the carotid blood in normal and HT guinea-pigs.

No. of animals columns:				Normal values		H-killed normal animals		H-killed HT animals-non-resistant		H survived HT resistant animals	
1	3	5	7	1 O_2 %	2 CO_2 %	3 O_2 %	4 CO_2 %	5 O_2 %	6 CO_2 %	7 O_2 %	8 CO_2 %
XI	16	1	26	19.5	28.8	0 36	65.6	4.55	55.2	4.88	49.4
IV	12	2	22	15.7	27.2	0.18	48.8	3.35	57.2	10.10	47.1
XV	13	24	19	12 9	36.1	0.41	63.1	0.98	47.5	8.62	43.5
VIII	14	28	16	17.9	28.1	4.26	50.6	2.45	55.2	9.66	40.2
XVIII	15	17	41	12.7	29.4	0.51	63.1	2.95	51.0	15.82	38.7
XVI		11	3	10.6	35.0			0.96	49.7	6.17	35.1
XIV		40	23	15 8	28.3			1.28	65.1	13 92	32.2
XII		9	11	19.7	38.7			1.46	44.2	11.50	31.9
		12	10					6.54	40.0	16.20	42.2
		40	14					4.02	34.8	13 60	31.4
		15						7.14	33.2		
		8						4.60	45.6		
		18						3.80	36 8		
		16						1.81	35.6		
No. of cases				8	8	5	5	14	14	10	10
$M = \frac{\Sigma x}{n}$				15.63	31.50	1.14	59.00	3.28	47.70	11.14	32.29
$\mu = \frac{\Sigma dx}{n} . 1{\cdot}25331$				3.35	4.83	1.57	8.96	2.00	9.72	3. 87	7.14
$\mu_x = \frac{\mu}{\sqrt{n}}$				0.94	1.71	0.74	4.02	0. 53	2.59	1.38	4.61

As is seen from Table I., in the normal animal death ensues when the O_2 content of the carotid blood falls from 15. 63 vol% to 1.14 vol%; i. e., when the blood contains practically no O_2 (Table I. column 3.).

Attention is to be called here to the circumstance that in this average in 4 out of the 5 cases the O_2 content did not reach 0, 6%, but in one of the 5 cases it attained 4.26%.

When HT guinea pigs died in consequence of s. c. H injection, the average O_2, content is 3.28 vol%. The significance of the difference (k) between these two values is 5.03. It is therefore certain that the HT animal which did not become more resistant to the lethal effect of H is more sensitive to the O_2 lack than the normal. The most striking difference is to be seen in the group of the animals which developed resistance to the lethal dose of s. c. given H. Here the average O_2 content is 11.14%, i. e., 70% of the normal 15.63% value. We can safely conclude that the HT animal when it becomes more resistant does not succumb to the normal s. c. 100% LD of H, because some change allows a better oxygenation in the lungs than that which takes place either in the normal or in the non-resistant HT animals. It is to be remembered that the HT animal, when its resistance has not been increased, dies at a higher O_2 content of the arterial blood than does the normal animal, but even this decreased resistance to the lack of O_2 is well overcompensated by the great amount of O_2 in the blood leaving the lungs.

On the passage of blood through the pulmonary circulation in the normal and HT animals killed by H we made the following observation: When blood is collected from the carotid of the normal guinea pig, the time between 2 drops is measured in seconds. The time required between 2 drops of blood in the normal animal at the peak of an H poisoning is 10 times as long. About the same time is necessary in the non-resistant HT animals, while in the resistant HT guinea pigs the time approaches the value obtained in the normal non-H-poisoned animal. It has been proved by FIELD and DRINKER (1930) that histamine causes in the guinea pig a contraction of the pulmonary arterioles. Since the output of the left ventricle increases in the resistant HT animals, we suppose that in these cases H does not cause the same constriction it does in the normal or non-resistant HT animals.

This increased oxygenation in the resistant HT animals may be due to an unaltered broncho-constrictor reaction to lower H level in the blood, or to a tardier, broncho-constriction to the same H level in the blood reaching the lung.

A lower blood level of H may be the consequence of a slowing down of adsorption. As Table II shows, the adsorption from the peritoneal cavity is almost unaltered in the HT animals, though there is a tendency in them to slower absorption.

It is clear from these values that there is very little slowing down of the adsorption in the HT animals. Attention must be drawn to the

circumstance that the standard deviation of the mean value of adsorption in the HT animals is not greater but smaller than that of the normal controls. This is significant because only part of the HT

TABLE II.

The absorption of H from the abdominal cavity in normal and HT guinea-pigs following the i. p. injection of 0.65 mg/100 g. b w. of H.

No.	Total injected µg	Remained in the peritonial cavity µg	Absorbed µg	dx	Remained %	Absorbed %	dx
88	2 230	234	1.996	117	10.4	89 6	5.6
70	3.000	600	2.400	287	20.0	80 0	4 0
75	2 300	240	2 060	53	10.4	89.6	5 6
77	2 500	440	2.060	53	17.6	82 4	1 6
82	2 660	272	2.388	275	10.2	89 8	5.8
73	2 030	368	1.662	451	18.1	81.9	2.1
83	2.600	448	2 152	39	17.2	82 8	1.2
74	2.350	560	1.790	323	24.2	75 8	8.2
81	2 350	324	2.026	87	13 7	86 3	2 3
71	2 600	264	2 336	223	10.1	89 9	5 9
79	2.800	368	2 432	319	13 1	86 9	2.9
95	2 850	800	2 050	63	27 0	73 0	11.0
			M_1=2,113	191		M_I=84	4 7
	Norma	control animals					
19	2.500	164	2 336	128	6.6	95.4	6.1
22	3.100	216	2 884	676	7.0	92.7	3.7
28	2.230	171	2 059	149	7 6	92.4	3 1
26	2.850	220	2.630	422	9.4	90 6	1 3
27	2.730	360	2.370	162	13.2	86 8	2.5
16	2.030	222	1 808	400	10.9	89.1	0.2
21	2.340	186	2 154	54	7.9	92 1	2.8
22	2.340	217.6	2.122.4	86	9.2	90.8	1.5
17	2.340	108.5	2 108 5	23	4.6	95 4	6.1
18	2.600	240	2.360	152	9.2	90.8	1.5
29	2.210	235 2	1 974 2	232	10 6	89.4	0 1
23	2.350	780	1 570	638	33.6	33.4	23.1
			H_2=2 208	260		M_{II}=89;3	4 33
	M_1-M_2=95 µg				M_I-M_{II}=5.3%		
	μ_1=239.1 ; μx_1=70.3				μ_I =5.84 ; μx_I =1 71		
	μ_2=325.1 ; μx_2=95.2				μ_{II}=5.43 ; μx_{II}=1.59		
	k (M_1 M_2)= 0.599				k (M_I M_{II})=2.26		

animals develope resistance — as shown in the previous paper. Others retain their normal sensitivity. It must be emphasized at this juncture that these 12 animals were those which survived a very energetic s. c. HT at the end of which the last dose was the 100% s. c. LD. They

were therefore more or less resistant. If this increase in the resistance in a few cases was due to a retarded adsorption, this latter should show in a few cases an outstanding slowing down, and this in turn should cause an increased standard deviation. However there were no animals in which the velocity of adsorption was outstandingly slowed down.

These are results of adsorption from the peritoneal cavity, whereas we made our O_2 and CO_2 determination in animals which had been poisoned by s. c. injections. Thus we have to suppose that the changes which took place during the HT in the capillary walls, in the venous circulation, in the colloid osmotic pressure and in other factors influenc-

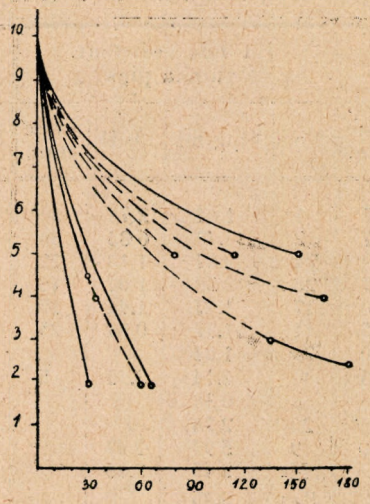

Fig. 1. The H content of the blood-plasma in normal and in HT animals after the injection of 10 μg/ml plasma H i. c. x-axis time in seconds after the i. c. injection of H, y-axis H content μg/ml blood plasma.

ing adsorption are the same, both in the peritoneal and subcutaneous circulation. If this is true we can expect that the change in the rate of adsorption is also the same in both cases; i. e., if there is a slowing down of H adsorption the same change takes place also in the sub-cutis.

The results of the experiments on the rate of disappearance of i. c. injected H from the arterial blood are shown in Figure 1. In this Figure the abscissa is the time in secs. elapsed between the i. c. injection of 10 μg/ml blood plasma of H and the withdrawal of blood sample from the left heart. The ordinate is the concentration of H in

μg/ml of blood plasma. As is seen in this Figure, the concentration of H decreases in the arterial blood very rapidly; the value of 2—5 μg/ml is reached within 30—165 secs. The values of normal and HT animals agree very well. As far as these 2x4 determinations go, there is no difference between the rate of disappearance of H from the blood between the normal and HT animals. The rate of adsorption being irregular and slightly reduced, the rate of disappearance from the blood remaining unaltered, one expects in the HT animals an irregular and slight reduction in the level of the blood H after s. c. injection.

TABLE III.

The H content of blood plasma of normal and HT guinea-pigs at the top of the symptoms of H poisoning following the s. c. injection of the 100 % L D

Normal guinea pigs				HT non resistent guinea-pigs				HT resistent guinea-pigs			
No.	Hμg/ml	O_2/H	Time min.	No.	Hμg/ml	O_2/H	Time min.	No.	Hμg/ml	O_2/H	Time min.
1.	5.92		7	1	1.64	2.78	30	26	2 62	8.94	30
2.	3.47		27	2	0.49	6.83	30	22	3.68	2.88	30
3.	0 88		33	24	0.87	1.10	22	19	1.48	5.83	30
4	2 02		17	28	2.28	1.07	15	16	3.28	2.94	30
5.	2 32		20	17	1.97	1.44	15	4	1.15	13.60	30
6.	4.59	0 06	15	11	1.97	0.84	25	3	1.24	4 96	15
11.	5 28	0 05	30	20	1.64			18	4.96		
12.	8 58	0,02	35	40	1.14	3.53	5	23	5.02	2.78	30
13.	13 12	1.57	20	6	0.38			38	12.10		
14.	2 71	0.27	25	25	2 26						
15.	2.46	0.40	10	9	5.92	2.47					
M_1	4.65	0.49		M_2	2,45	2.46		M_3	3.91		
μx_1	0.98			μx_2	0.45			μx_3	0.94		
k_{1-2}	2.00			k_{3-2}	1.39			k_{1-3}	0.54		

Finally, in Table III. we present our H estimation in the blood of normal and HT animals.

The calculation of the significance of difference between the normal lethal level of H in the arterial blood and the level of H in the blood of those HT animals which resisted the 100% s. c. LD shows that these 2 values agree very well (k_1—k_3=0.5). The slight tendency towards a decreased H concentration is in accordance with the slight tendency to a slowing down in the adsorption of H into the blood. These experiments provide clear-cut evidence in favour of the mechanism in which the resistance towards this s. c. LD of H is due to better oxygenation of the blood in the lungs in the presence of the LD of H. This con-

clusion becomes even more evident if we compare the vol% of $O_2/\mu g$ H values. Here we see this value is 0.4 for the normal, whereas for the resistant HT it is 6. The oxygenation of the blood in the resistant HT animal per unit weight of blood H is therefore 15 times greater than in the normal one. It is evident that even in those cases where the slowing down of the adsorption of H into the blood took place and resulted in a reduced blood H this plays only an inferior role in the mechanism of resistance. Considering the mechanism of death and H content in the HT non-resistant animals, we saw that these animals oxygenate much better than do the normal ones. (3.27 vol% O_2 as

TABLE IV.

The lactic acid content of the blood of normal and HT animals during the symptoms of H poisoning

	Normal	HT
No. of cases (x)	10	8
$M = \frac{\Sigma x}{n}$	32 mg%	46 mg%
$\mu = \frac{\Sigma d x}{n} \cdot 1{\cdot}25331$	9·6	11·4
$\mu_x = \frac{\mu}{\sqrt{n}}$	3·	4·
$k = \frac{M_1 - M_2}{\sqrt{u_1^2 + u_2^2}}$	2·8	

against 1.14%). Their blood H with its average of 2.45 μg/ml. is the smallest among the 3 groups, but the significance of difference is very low in both combinations. The quotient O_2 vol%/H is 2.47, the non-resistant HT animal oxygenates 6 times better than the normal ones. But it is obviously more sensitive to the lack of oxygen, since it dies at the average value of 3.28%. The normal dies as a rule at 0.4%, and only occasionally at 4.26%. Though the lung oxygenates better, it does not do so at a sufficiently good rate to raise the O_2 content of the blood high enough to satisfy the increased O_2 hunger of the tissues. This is the case only in the resistant HT animal.

Passing to the lactic acid determination, we see in Table IV. that the blood of the HT animals contains more lactic acid than does the normal one.

As regards the CO_2 content of the blood, we see in Table I. that it is elevated as compared to the normal value in all H-poisoned animals and is normal in the resistant HT animals. The acidaemia is clearly smallest in the group where the bronchoconstrictors relax the most — in spite of the presence of H; consequently the relaxation cannot be due to acidaemia.

Some clue to the nature of the changes brought about by the HT may be gained from the post mortem examinations. The results recorded here have been obtained partly on animals whose details, such as mode of HT, lethal dose, length of survival, etc. have been recorded in our previous paper (Beznák, Kovách, Gáspár-Rády 1947).

In the 3 kinds of animals: normal, non-resistant HT and resistant HT all killed by H, 2 types of situs have been observed.

The following description gives a picture of the unmixed clear-cut types. Combinations of the different symptoms have occurred.

a.) First Type: Seen in the acute histamine poisoning of the normal animal.

In the heart the auricles, especially the right one, were filled to the maximum with blood; out of the ventricles the left one was found in systole containing but little residual blood. The right ventricle, on the other hand, was dilated to its utmost capacity with much blood, imitating diastole. The coronary venae were full of strongly reduced blood. *The lungs* were non-collapsible, dilated to the maximum, fairly congested, slightly edematous. (Auer-Lewis). *The abdominal organs, the liver,* but for varying degrees of congestion (dilated venae centrales), appeared normal. Turbidity or fatty degeneration of the parenchym was not perceived. *The intestines* showed all signs of congestion with occasional small bleedings. Apart from very little pronounced venous congestion we found no change in *the kidneys.* The *suprarenal cortex* was obviously hyperaemic with arterial blood. It is to be noted that the symptoms are always equally severe whether death was caused by a small or by a large dose.

b.) Second Type: Seen in animals whose resistance had not been increased by the HT.

Whereas the normal animals die within 20 minutes after the s. c. or i. p. H injection, out of the 13 non-resistant animals 9 (70%) died only several hours after the injection. At autopsy the picture of *the heart* appeared to be similar to that seen in the first type animals, though perhaps turbidity could be seen. In *the lungs* infarcts have been seen regularly; their size varied from the size of a bean to such an

extent that one or several of the lobes became indistinguishable from the pneumonia "hepatisatio rubra" stage. Those parts of the lungs which had not been affected showed the usual AUER-LEWIS type. Signs of severe chronic congestion were present in *the liver* in all cases; turbidity and fatty degeneration were regular. *The intestines* were not so full of blood as in Type I, yet the signs of chronic congestion were apparent. They were empty and atonic.

The distribution of the two types within the HT animals, Groups I—VI (first paper) was as follows: The second type was mostly seen in Group IV. Out of the 38 autopsied animals in this group 13 died from doses 80—130 μg/100 g during the first part of the HT. In 12 of the 13 (92%) the typical severe symptoms in the lungs were seen. From the 25 HT animals which died later from larger doses in only 14 cases (56%) were infarcts observed. It is significant that they were never as extensive as in the case of animals which died from smaller doses. Severe liver changes were seen in 46% of the sensitive HT and 12% of the other animals.

Between the situses of the *normal* and the *resistant HT animals* (i. e., those killed by larger than the normal 100% lethal dose) killed with H, only one marked difference was found at autopsy. The general picture in these was that of an acute H-poisoning (Type I) but in the HT animals on a background of *chronic venous congestion,* without the severe changes of the parenchyms of the organs.

If we compare the results of the autopsies, it is clear that in those non-resistant HT animals which died from small H doses the symptoms of a chronic venous congestion, and especially its consequences in the parenchym of the heart, lungs and liver were much more pronounced than in the case of either the normal or the resistant-phase animals. Besides this severe chronic venous congestion, the almost regular (12 out of 13) appearance of extensive pulmonal infarcts distinguished non-resistant animals from both the normal and the resistant ones.

DISCUSSION.

The discussions of the O_2 content of the arterial blood of the normal, non-resistant HT and resistant HT guinea pigs showed that in the normal animal at the height of a mortal H-shock provoked by a 100% LD, the O_2 drops from the physiological level of 16%, to 1%; in the non-resistant HT animal to 3%. In the resistant HT

guinea pig during or after the shock provoked by the 100% LD — which, however, these animals survived — the O_2 content sinks only to 11%. These figures warrant the conclusion that a link in the chain of life-processes, which enable some HT animals to resist the normally 100% LD of H, is that HT brings about some change in the organism of the guinea pig in consequence of which better oxygenation takes place in the lungs. Since following a s. c. injection of H in the normal and in the non-resistant HT and in the resistant HT animals, the H level in the blood at the height of the shock does not show a significant difference, yet in the normal practically no, in the non-resistant HT hardly any, and in the resistant HT animals fairly good oxygenation takes place, it follows that the change brought about by the HT consists in enabling the lungs to oxygenate better in spite of the presence of such a concentration of H in the blood as makes this oxygenation otherwise impossible. (The slight and irregular tendency to a slowing down of the adsorption from the site of injection in the HT animals accords well with the small and irregular tendency to a reduced level of blood H, but their role in the resistance is insignificant.) As regards the nature of the change enabling the lungs to oxygenate better, caused by HT we know that it can only be a smaller contraction of the bronchioles and of the arterioles (and capillaries?), of the pulmonary circulation. On the question why these HT smooth muscles contract less vigorously to the same H dose than the normal ones do, the following circumstances give some indication: The diminished responsiveness cannot be due to acidaemia alone, though it is true that Eichler *et al.* have shown that acidaemia relaxes the bronchoconstriction, and we found an increased concentration of lactic acid in the HT animals. On the other hand it is also true that the CO_2 tension during the shock is highest in the normal animal which dies, and lowest in the HT animal that survives.

Besides incompensated acidaemia some other changes in the organism or in the smooth muscles in the lungs themselves, must take place, in the HT animals only.

From the facts known so far the following hypotheses are feasible: Adrenaline is known to relax the histamine contraction of the bronchioles (Cameron and Tainer 1936, Daly, Foggie and Hebb, 1940). Evidence of suprarenal hypertrophy (at any rate of the increase of the suprareral/b. w. ratio) in the HT animals is produced (MacKay and Clark 1938). M. Beznák, Korényi and Hajdu (1942) provided experimental proof that acidaemia causes a hypertrophy of medulla and cortex. Korényi and

HAJDU (1942) showed, however, that hypertrophy of the adrenals is not always accompanied by hyperfunction. It is therefore possible that in the diminished response of the smooth muscle in the lung, hyperadrealinaemia concommittant to the acidaemia is the additional factor which only exists in the HT animal, and not in the normal.

It is known that a number of imidazole derivatives, especially arginine are antagonistic to H (ACKERMANN, 1939). HT causes profound changes in the protein metabolism, thus it is possible that amongst the intermediary metabolites such antagonists are liberated.

A very well established fact is that suprarenalectomy increases, and suprarenal cortical hormone decreases, the toxicity of H, and, as we mentioned, there is a suprarenal cortical hypertrophy in the HT animal. But what we know of the mode of action of the suprarenal cortex in counteracting the effect of H, does not support the assumption that it decreases the responsiveness of the smooth muscles to the lung. As opposed to this, we know that it is necessary to the normal contractibility of the arterioles and capillaries.

Yet another hypothetical possibility is that HT damages the smooth muscle of the bronchioles and arterioles, in the lungs, in consequence of which damage they simply cannot contract to H as vigorously as the normal ones do. This assumption is supported by the general failing appearence of the HT animals, by the infarcts and edema seen at autopsy chiefly in the non-resistant HT animals, and by the histological findings. (HEINLEIN, 1937, MERKEL, 1942, JANCSÓ, 1941, TÖRŐ, 1942). These latter indicate severe damage in the blood vessels of the lungs and the heart, as well as in the heart muscle. Although so far this hypothesis seems to be the most plausible, it must be pointed out that these damages, such as infarcts, edema etc., were less pronounced in the resistant than in the non-resistant HT animals. It is of course possible that the damage caused by HT was in the resistant animal just enough to decrease the responsiveness of the resistant HT smooth muscle and in the non-resistant ones it went further, thus reducing the functioning surface of the lungs with the severe haemorrhages.

All these are, however, mere speculations. Only further experiments can decide which of them or yet another, hitherto unforseen, mechanism is the true cause of the decreased responsiveness of the bronchioles and arterioles in the resistant HT animals.

In conclusion, we must warn against any generalisation. The mechanism of H death in other species is different from that in guinea pigs. In those animals therefore, where the H killed them through other me-

chanism than broncho-constriction the HT may not increase the resistance (Remember that CSÁKY and KOVÁCH proved that the HT does not alter in the cat the blood pressure response to H). Moreover, it may be even in the guinea pig that death through bronchoconstriction-asphyxia is only the most general mechanism, but that in some other cases some other vital function breaks down. This may, e. g., be the failure of the right heart. It is possible that in this mechanism HT will have no life-saving effect. Perhaps such animals form the group of guinea pigs whose resistance could not be increased by HT.

SUMMARY.

1.) Comparative determination of the O_2 contents of guinea pigs' blood proved that the cause of the resistance to a s. c. injected LD of H in the resistant HT animals is better oxygenation.

2.) This better oxygenation is due to a decreased contraction response of the broncho- and vaso-constrictor muscles to the same level of H in the blood, since determination of the blood in normal, non-resistant HT guinea pigs showed no significant difference.

3.) The small and irregular relative diminution of the blood H after s. c. H injections, in the HT animals accords well with the small and irregular slowing down of adsorption of H from the site of injection (peritoneal cavity) and with the circumstance that i. c. injected H disappears from the blood equally fast in both normal and HT animals.

4.) The blood lactic acid content of the HT animals is higher than that of the normal ones. During H shock the CO_2 content of the blood is highest in the normal, and lowest in the resistant HT animals. The diminished contraction of the smooth muscles in the lungs cannot therefore be conditioned solely by the acidaemia.

5.) The possible physiological mechanism of the decreased responsiveness caused by HT is discussed. It is pointed out that at present the likeliest explanation is that HT damages the broncho- and the vaso-constrictor muscles which consequently are unable to contract and allow a better oxygenation, and increased blood flow through the lungs, This hypothesis is supported by the autopsy results.

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REFERENCES.

ACKERMANN, D. (1939). *Naturwiss.* **515.**

BARSOUM and GUDDUM, J. H. (1935). *J. of Physiol.* **85.** 1.

BEZNÁK A., KOVÁCH, A. Gy. B., GÁSPÁR-RÁDY, Zs. (1947) *Arch. Biol. Hung.* **17.**

BEZNÁK, M., KORÉNY Z. and HAJDU, I. (1492). *Arch. Intern. Pharm. Therap.* **67.** 242.

CAMERON, W. M., and TAINER, M. L. (1936). *J. of Pharmacol.* **57.** 152.

CODE, C. F. (1937). *J. of Physiol.* **89.** 257.

CSÁKY, T., KOVÁCH, A. (1946). *Arch. Biol. Hung.* **17.**

DALE, H. H. and LAIDLAW, P. P. (1910—11). *J. of Physiol.* **41.** 318.

DALY, DE BURGH I., FOGGIE, P. and HEBB, C. C. (1940). *Quart. J. of Physiol.* **30.** 21.

FICHLER, O., and MÜGGE, H. (1931). *Arch. exp. Path. u. Pharmak.* **159.** 633.

EICHLER, O., and KILLIAN, H. (1931). *Arch. exp. Path. u. Pharmak.* **159.** 606.

EICHLER, O., SPEDA, G. (1940). *Naunyn-Schiedebergs Arch.* **195.** 102.

FIELD, M. E., and DRINKER, C. K. (1930). *Amer. J. Physiol.* **93.** 138.

GREEN, H. H. (1945). *Brit. Med. Bull.* **3.** 692.

HEINLEIN, H. (1937). *Zbl. Path.* **66.** 93.

JANCSÓ, M. (1941). **11.** *Tagung. Ung. Physiol. Ges. Physiol. Ber.* **126.** 475.

KARÁDY, St. (1935). *Arch. exp. Path. u. Pharm.* **180.** 282.

KARÁDY, St. (1938). *Amer. J. Physiol.* **123.** 194.

KARÁDY, St., SELYE H. and BROWNE, J. S. L. (1938). *J. of Immunol.* **35.** 335.

KOKAS F., SARKADY, L., WENT, I. (1938). *Magy. Orv. Arch.* **39.** 408.

KORÉNYI, Z., HAJDU, I. (1942). *Arbeitsphysiol.* **12.** 31.

LAURENSEN, F., and WAHLLÄNDER, H. W. (1938). *Biochem. Ztschr.* **298.** 273.

MACKAY, E. M., and CLARK, W. G. (1938). *Proc. Soc. Exp. Biol. and Med.* **39.** 56.

MERKEL, H. (1942). *Ztschr. exper. Med.* **111.** 98.

NOBLE, R. L., and COLLIP, J. B. (1941). *Amer. J. Physiol.* **133.** 633.

ROSE, B., KARÁDY, St., BROWNE, I. S. L. (1940). *Amer. J. Physiol.* **129.** 218.

RUSZNYÁK, St., KARÁDY, St., SZABÓ, D. (1936). *Arch. f. klin. Chirurg.* **187.** 279.

SELYE, H. (1937). *Science* **85.** 247.

VAN SLYKE, D. (1927). *J. Biol. Chem.* **74.** 659.

TÖRŐ, I. (1942). *Ztschr. mikr.- and Forsch.* **52.** 552.

WILSON, H. (1941). *J. of Physiol.* **94.** 241.