ON THE SYNTHESIS OF ANEURINPYROPHOSPHATE FROM ANEURIN BY BLOOD

by

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According to observations by OCHOA [1], which we could fully confirm [2], the aneurinpyrophosphate (APP) content of kidney and liver slices of aneurin deficient animals (rats and pigeons) increases, when the slices are incubated for some hours in an aneurin solution, but the level reached is never higher than the value found in animals that were well provided with aneurin. We now investigated the synthesis of APP by blood of various species, to which aneurin had been added.

The APP determinations were carried out according to a manometric method, published previously [3]. In general we employ 2 cm³ blood for a determination, but the accuracy is only slightly affected, or, if the APP content is 10 γ per 100 cm³ or more, not affected at all when 1 cm³ is used. Hence, when the volume of blood at our disposal was small, 1 cm³ was used for a determination.

The blood of rats and guinea pigs was drawn off by introducing a glass canula into the aorta. The blood of oxen, calves, horses, sheep, and pigs was obtained from the slaughterhouse and usually investigated immediately after it had been transferred to the laboratory; only in a few cases we were compelled to store it in the refrigerator for 1 or 2 days. The chickens were decapitated and the blood was collected in a beaker containing some drops of a concentrated Na oxalate solution. The human blood was drawn off by punction of the elbow vein. The blood of the frogs was drawn from the heart. The amount of frog blood obtained was too small to be examined in the common way. Therefore we had to take refuge to LINDERSTRØM-LANG'S diver technique. The same technique was employed in a few experiments on the formation of APP in blood *in vivo*.

The first experiment was carried out with blood of aneurin deficient rats. Normal rats' blood contains about 15 γ APP per 100 cm³. As Table I shows, the APP content of the blood of the deficient rats was very low before incubation, but was raised to a value much above the normal level by treatment with 24 γ aneurin per cm³ for 1 to 2 hrs at 37° C.

TABLE I

INCUBATION OF BLOOD OF ANEURIN DEFICIENT RATS WITH 24 γ PER CM³ AT 37°

Rat No.	Hours of incubation	γ APP per 100 cm³ blood	
		before incubation	after incubation
1	1	0.5	28
2	1.5	0.5	52
3	2	0.5	52

Hence it seemed probable that the content of normal rats' blood might also be increased by incubation with aneurin, possibly to still much higher values by adding

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more aneurin and incubating for a longer time. Indeed, as Table II shows, the APP content of normal rats' blood could be increased to more than 100 γ per 100 cm^3 by incubating it with 1 mg aneurin per cm^3. The maximum was reached after 4 hours' incubation, after which the APP content declined again.

TABLE II

INCUBATION OF NORMAL RATS' BLOOD WITH 1 MG ANEURIN PER CM^3 AT 37°

Hours of incubation	γ APP per 100 cm^3 blood		
	Rat No. 4	Rat No. 5	Rat No. 6
0	11.3	—	20.2
1/4	22.5	—	—
1/2	27.0	—	—
1	36.0	—	69
2	56.0	85	97
4	—	117	126
6	—	114	126
8	—	59	115

The amount of APP formed increases very rapidly with the amount of aneurin added; the maximal amount is formed when 1 mg aneurin is added to 1 cm^3 blood (see Table III). Hence, though not knowing if the same rules would hold for the blood of other species, the blood samples were always incubated for 4 hours at 37° C with 1 mg aneurin per cm^3 when we started a comparison of the APP synthesis by various kinds of blood in vitro.

In these experiments one sample of the blood was deproteinized without having been incubated, a second sample was incubated for 4 hours with 1 mg aneurin per cm^3 and a third sample was incubated without adding aneurin.

TABLE III

INCUBATION OF NORMAL RATS' BLOOD WITH VARIOUS AMOUNTS OF ANEURIN FOR 4 HOURS AT 37°

mg aneurin added per cm^3 blood	γ APP per 100 cm^3 blood	
	Exp. 1	Exp. 2
0	14.2	15.7
0.001	26.2	—
0.010	53.0	—
0.100	100	74
1	115	108
2	—	108
4	—	108

In the first place we investigated another 4 samples of normal rat blood. The results are given by Table IV. The data collected in this table confirm the results recorded above. Besides the increase by incubation with aneurin a decrease of about 20 to about 40 pCt of the APP is observed in the samples incubated for 4 hours without aneurin.

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In our next experiment, carried out with human blood, we found no rise of the APP content at all, though the blood was drawn from patients with a rather low APP

TABLE IV

INFLUENCE OF INCUBATION AT 37° WITH OR WITHOUT ANEURIN ON THE APP CONTENT OF NORMAL RATS' BLOOD

	γ APP per 100 cm³ blood			
Rat No.	7	8	9	10
Before incubation	19.1	20.2	22.5	15.8
Incubated 4 hrs with 1 mg aneurin per cm³	81	99	107	109
Incubated 4 hrs without aneurin	12.4	12.4	12.4	12.4

TABLE V

INFLUENCE OF INCUBATION AT 37° WITH OR WITHOUT ANEURIN ON THE APP CONTENT OF HUMAN BLOOD

	γ APP per 100 cm³ blood			
Patient	Kr.	Kl.	de J.	B.
Before incubation	4.1	5.6	6.7	7.3
Incubated 4 hrs with 1 mg aneurin per cm³	5.6	6.1	7.4	7.4
Incubated 4 hrs without aneurin	5.1	6.2	6.2	6.7

content of their blood. This forms a sharp contrast to rat blood. Normal human subjects rarely have a lower APP content than 7 γ per 100 cm³; 9 or 10 γ is a common result. Our data on human blood are given in Table V.

A second difference between human blood and rat blood is that, allowing for the error in the determination, the APP content of the first is not affected by incubation without aneurin either (comp. Tables IV and V).

TABLE VI

INFLUENCE OF INCUBATION AT 37° WITH OR WITHOUT ANEURIN ON THE APP CONTENT OF OX BLOOD AND CALF BLOOD

	γ APP per 100 cm³ blood						
	Ox blood			Calf blood			
	fresh		2 × 24 hrs. in refrig.	fresh		2 × 24 hrs in refrig.	
Ox No.	1	2	3	—	—	—	—
Calf No.	—	—	—	1	2	3	4
Without incubation	4.5	4.5	5.1	10.1	10.1	9.0	7.9
Incubated 4 hrs with 1 mg aneurin per cm³	8.0	9.9	7.5	31.0	26.5	32.0	14.0
Incubated 4 hrs without aneurin	2.8	4.5	5.1	10.1	9.0	8.4	8.4

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After we had established these differences between rat blood and human blood we were still more interested in the behaviour of other kinds of blood. Table VI gives the results obtained with ox blood and blood of new-born calves. We see that the APP content of the blood of the calves is higher than that of the blood of the oxen, just as we had found previously [3]. The increase after incubation with aneurin is only small in the case of the oxen, but much greater, though considerably less than with rat blood, in the case of the calves. The blood of one calf (No. 4) only showed but a slight increase, but possibly this was caused by storage in the refrigerator, though the blood of calf No. 3, which had been stored for the same time, showed a maximal increase. As will been seen in the experiments with pig blood, a few days storage in the refrigerator does not seem to be detrimental to the synthesizing power in general (see Table VIII). Incubation in the absence of aneurin does not notably affect the APP content of ox blood and calf blood.

The APP content of fresh horse blood and fresh sheep blood is low and the increase after incubation with aneurin only slight, while it is not affected by incubation without aneurin (see Table VII).

Pig blood, however, appeared to have a synthesizing power nearly as great as that of rat blood, while incubation without added aneurin did not affect the APP content (see Table VIII). Still larger synthesis, even surpassing that of rat blood, was exhibited by chicken blood, while the decrease after incubation without aneurin was negligible in this case. As chicken blood had not been investigated before, we also

TABLE VII

INFLUENCE OF INCUBATION AT 37° WITH OR WITHOUT ANEURIN ON APP CONTENT OF HORSE BLOOD AND SHEEP BLOOD

	γ APP per 100 cm³ blood			
	Horse blood fresh		Sheep blood 18 hrs in refrigerator	
Horse No.	1	2	—	—
Sheep No.	—	—	1	2
Without incubation	1.7	1.1	4.7	4.0
Incubated 4 hrs with 1 mg aneurin per cm³	1.9	5.0	11.2	10.9
Incubated 4 hrs without aneurin	2.3	1.7	3.4	4.4

TABLE VIII

INFLUENCE OF INCUBATION AT 37° WITH OR WITHOUT ANEURIN ON APP CONTENT OF PIG BLOOD

	γ APP per 100 cm³ blood					
	Fresh blood			48 hrs in refrigerator		
Pig No.	1	2	3	1	2	3
Without incubation	15.8	16.9	13.5	12.5	17.0	13.6
Incubated 4 hrs with 1 mg aneurin per cm³.	76	76	89	65	73	88
Incubated 4 hrs without aneurin	11.3	—	13.5	11.3	17.0	15.9

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give the APP content of 5 more fresh samples, non-treated with aneurin (see Table IX).

Frog blood also has a great synthesizing power, while this is totally lacking in the blood of the guinea pig, which already possesses a very high APP content. The data on these kinds of blood are collected in Tables X and XI. The blood of the frogs was incubated at 27° C instead of 37°.

The experiment with guinea pig No. 1 would indicate that a small synthesis has taken place, as incubation in the absence of added aneurin results in a decrease of APP, while the sample incubated with aneurin, though its increase may be called negligible, certainly has not decreased.

TABLE IX

INFLUENCE OF INCUBATION AT 37° WITH OR WITHOUT ANEURIN ON APP CONTENT OF CHICKEN BLOOD

	γ APP per 100 cm³ blood							
Chicken No.	1	2	3	4	5	6	7	8
Without incubation. .	11.0	—	8.1	5.6	6.1	6.6	7.5	7.2
Incubated 4 hrs with 1 mg aneurin per cm³	334	323	550	—	—	—	—	—
Incubated 4 hrs without aneurin . . .	8.1	8.1	7.3	—	—	—	—	—

TABLE X

INFLUENCE OF INCUBATION AT 37° WITH OR WITHOUT ANEURIN ON THE APP CONTENT OF BLOOD OF GUINEA PIGS

	γ APP per 100 cm³ blood	
Guinea pig No.	1	2
Without incubating	81	44
Incubated 4 hrs with 1 mg aneurin per cm³ . . .	88	44
Incubated 4 hrs without aneurin	62	44

TABLE XI

INFLUENCE OF INCUBATION AT 27° WITH OR WITHOUT ANEURIN ON THE APP CONTENT OF FROG BLOOD

	γ APP per 100 cm³ blood
Without incubating	9
Incubated 2 hrs with 1 mg aneurin per cm³	84
„ 4 „ „ 1 „ „ „ „	79

The data given in Tables I to XI demonstrate that there is a very wide variance in the behaviour of different kinds of blood. And moreover bloods capable of synthesizing APP from aneurin *in vitro* differ much from other tissues — as rats' and pigeons' kidney and liver —, also possessing synthesizing power : in the blood APP often accumulates to very high contents, bearing no relation whatever to the normal physiological APP level, while in slices of kidney and liver of avitaminous animals the APP content never rises above the normal value.

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We have tried to find some correlation between the capacity of blood to form APP *in vitro* and other properties concerning aneurin metabolism. We have formerly observed an equally wide variance in what we have called the formation of „temporary" APP [4], so some connection between both phenomena might exist.

With „temporary" APP we have denoted great amounts of APP, which accumulated in various tissues of the rat and the frog, and also smaller amounts in the tissues of the pigeon after injection of very high doses of aneurin, viz., 10 mg for the rat, 1 mg for the frog, 20 mg for the pigeon. After some days this APP has disappeared again notwithstanding the body of the rats and pigeons is amply supplied with aneurin from the food. The APP present under normal conditions was called „permanent" APP. Now there is a wide variance in the capacity of different species to form „temporary" APP, the rat and the frog possess this capacity in a very high degree, the pigeon to a much lower extent, while it seems to be absolutely lacking in the chicken, for temporary APP was not formed even after injection of 100 mg aneurin. We now found that it is also lacking in the guinea pig (injection of 30 mg ; see Table XII).

TABLE XII

APP CONTENT OF GUINEA PIG ORGANS *, BEFORE AND AFTER SUBCUTANEOUS INJECTION OF 30 MG ANEURIN

	γ APP per g			
	without injection		2 hrs after injection of 30 mg aneurin	
Guinea pig No.	1	2	3	4
Heart **	9.7	9.0	8.6	9.2
Liver	6.0	5.0	8.8	9.6
Kidney	10.0	9.0	11.2	11.0
Diaphragm **	5.7	4.6	3.9	4.8
Leg muscle **	3.3	2.4	2.3	3.5
Brain	3.0	2.8	2.3	2.6

* Method of determination, see 5). ** Just as we found previously for other species APP content of heart muscle > APP content of diaphragm > APP content of leg muscle.

Though we were not able to do any experiments with pigeon blood as these animals were absolutely unobtainable at the time when this work was carried out, we can conclude from comparing the behaviour of the other kinds of blood with the capacity for forming temporary APP by the corresponding species that there is no connection between both properties. The frog and the rat both possess the property of forming temporary APP in a high degree, while their blood also synthesizes much APP *in vitro*. On the other hand the chicken does not form any temporary APP, while the capacity of its blood for synthesizing APP in vitro is the highest of all kinds of blood investigated. The guinea pig does not form temporary APP either but in this case the blood also lacks all power of APP synthesis *in vitro*.

We have also performed a number of experiments on the influence of intravenous injection of large doses of aneurin on the APP content of the blood *in vivo*. The amounts of aneurin injected were proportional to the body weights of the animals. The APP determinations were carried out by means of the diver method in

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10 to 25 mm³ blood. In the three species investigated, rat, chicken, and guinea pig, we found a considerable increase of APP, even in the case of the guinea pig. This APP is „temporary" APP of the blood, for it disappears again in the course of time, notwithstanding ample provision with aneurin from the food. We know that the APP content of guinea pig blood is unaffected by incubation with aneurin *in vitro*. And while the increase in rat blood *in vivo* was about equal to the increase *in vitro*, the increase in chicken blood *in vivo* was much smaller than the increase *in vitro* (see Table XIII). So there is no correlation between the formation of APP by blood *in vitro* and the formation of „temporary" APP in other tissues or of the „temporary" APP of blood.

TABLE XIII

INFLUENCE OF SUBCUTANEOUS INJECTION OF VERY LARGE DOSES OF ANEURIN ON THE APP CONTENT OF BLOOD

Time after injection	γ APP per 100 cm³ blood			
	Guinea pig		Rat	Chicken
	(Injection of 30 mg)		(Injection of 10 mg)	(Injection of 20 mg)
	I	II		
0	27	50	18	8
1 hr	—	—	56	65
2 hrs	94	80	90	54
4 „	—	—	—	90
5 „	—	—	75	—
8 „	—	—	—	90
24 „	—	—	75	38
2 days	—	—	—	45
4 „	—	—	23	—
6 „	—	—	—	25

SUMMARY

1. The blood of various species exhibits great differences in the capacity for synthesizing aneurinpyrophosphate (APP) from aneurin *in vitro*. While in human blood and the blood of guinea pigs this capacity is completely lacking, chicken blood, rat blood, pig blood and frog blood possess it in a very high degree. After incubation with 1 mg aneurin per cm³ for 4 hours at 37° C the APP content of a sample of one of the latter kinds of blood surpasses the normal level several times. In ox blood, sheep blood and horse blood the formation of APP is only small. The APP content of calf blood is distinctly higher than that of ox blood and it has a much greater synthesizing power.

2. No correlation could be found between the capacity for forming APP *in vitro* and the formation of „temporary" APP in blood and other tissues (*i.e.*, APP formed after injection of large doses of aneurin into the living animals. In contrast to the „permanent" APP of the tissues, this APP disappears again in the course of one or more days, notwithstanding the animals' diet is well provided with aneurin).

3. The aneurinpyrophosphate content of a number of organs of the guinea pig was determined.

RÉSUMÉ

1. Le sang de diverses espèces d'animaux accuse de grandes différences en ce qui touche la synthèse du pyrophosphate d'aneurine (APP) *in vitro*. Tandis que le sang de l'homme et le sang du cobaye ne possèdent nullement cette propriété, le sang

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de la poule, du rat, du porc et de la grenouille la possèdent à un très haut degré. Après une incubation de 4 heures à 37° C avec 1 mg d'aneurine par cm^3, la teneur en APP d'un échantillon de l'une des sortes de sang précitées dépasse de plusieurs fois le niveau normal. Dans le sang de boeuf, de mouton et de cheval, la formation d'APP est très faible. La teneur en APP du sang de veau est visiblement plus élevée que celle du sang de boeuf et il possède un pouvoir synthétique beaucoup plus grand.

2. Aucune corrélation n'a pu être établie entre la propriété du sang de former APP *in vitro* et la formation d'APP „temporaire" dans le sang et dans d'autres tissus (c. à. d. d'APP formé après injection de fortes doses d'aneurine dans le corps des animaux vivants. Contrairement à ce qui a lieu pour l' APP „permanent" des tissus, cet APP disparaît dans l'espace d'un ou de deux jours, bien que la nourriture contienne de l'aneurine en abondance).

3. La teneur en pyrophosphate d'aneurine d'un certain nombre d'organes du cobaye a été déterminée.

ZUSAMMENFASSUNG

1. Das Blut verschiedener Arten weist grosse Unterschiede bezüglich des Synthesevermögens von Aneurinpyrophosphat (APP) aus Aneurin *in vitro* auf. Während Menschenblut und Meerschweinchenblut dieses Vermögen vollkommen entbehren, besitzt das Blut des Huhnes, der Ratte, des Schweines und des Frosches diese Fähigkeit in hohem Masze.

Nach Inkubation mit 1 mg Aneurin pro cm^3 während 4 Stunden bei 37°C übersteigt der APP Gehalt einer Probe der vorerwähnten Blutsorten den normalen Gehalt um das Vielfache.

Im Ochsen-, Schafs- und Pferdeblut ist die APP-Bildung nur gering. Der APP-Gehalt des Kalbsblutes ist grösser als der des Ochsenblutes und das Synthesevermögen des ersteren ist viel grösser.

2. Es besteht kein Zusammenhang zwischen der Bildung von APP durch Blut *in vitro* und der Bildung von „temporarem" APP (d.h. von APP, das infolge der Injektion von grösseren Dosen Aneurin in den Geweben von lebenden Tieren gebildet wird. Im Gegensatz zun „permanenten" APP der Gewebe verschwindet dieses APP innerhalb von ein oder zwei Tagen, obgleich das Futter der Tiere reichlich Aneurin enthält).

3. Der Aneurinpyrophosphatgehalt ist für eine gewisse Anzahl Organe des Meerschweinchens bestimmt worden.

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Received March 26, 1946.