reproduire par un réflexe conditionnel les actions pharmacologiques exercées sur les organes effecteurs[3]. Ainsi, OBÁL et al.[2] n'ont pas obtenu de l'hyperthermie conditionnelle en utilisant le 2,4-dinitrophénol, tandis qu'ils ont réussi à conditionner l'hyperthermie amphétaminique.

Cette interprétation concorde avec les données[1,4] concernant les différentes actions centrales des drogues cholinergiques. Connaissant la relation fonctionnelle entre les centres thermiques et la formation réticulée[5] et tenant compte de l'importance de cette formation dans la génèse des réflexes conditionnels[6], nous admettons que c'est justement l'action de la carbaminoylcholine sur les cholino-récepteurs du système activateur ascendant réticulaire[4] qui déterminerait son activité thermolytique et le résultat de la réponse conditionnelle fixée à elle.

Zusammenfassung. Ein auf die Wirkung von Carbaminoylcholin an Ratten aufgebauter bedingter Reflex ruft eine Temperatursenkung hervor. Dieses Ergebnis weist auf eine auf den afferenten Teil der Formatio reticularis gerichtete Wirkung des Stoffes hin.

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Stabilization of DNA Structure by Histones against Thermal Denaturation[1]

Histones have been discussed in recent years as possible regulators of the function of genes, by blocking the surface of the desoxyribonucleic acid (DNA) to which they are attached[2,3]. Although the details of the structure of the native nucleoprotein are not yet known, it has recently been suggested that the histone alpha-helix fits into the 'large groove' of the WATSON-CRICK DNA double helix, at an angle of 60° to the main DNA axis, and that thus histone molecules could form bridges between DNA helices, or stabilize a long DNA helix into a 'super-helix' of as yet unknown dimensions[4]. In the course of our own investigation into the structural ageing of DNA, we have been led to the idea that histone, firmly bound to the nucleic acid in preparations from thymus of aged bovines, stabilizes it against thermal denaturation[5]. The present communication reports on the behaviour of nucleoprotein and DNA preparations with different amounts of residual histone (prepared from individual bovine thymus) on thermal denaturation, and presents evidence for the stabilizing effect of histones on the DNA double helix.

Material and methods. Starting with individual bovine thymus, the nucleoprotein (method of CRAMPTON et al.[6]) and DNA in two degrees of purity (method of KAY et al.[7], see also [5]) were prepared each time. Histone content of the DNA preparations was calculated from the determination of arginine content (method of SAKAGUCHI[8]), and phosphate was determined by the method of JONES et al.[9]. Thermal denaturation was measured in $0.0025\,M$ $NaNO_3$, pH 5.4, at a DNA concentration of about 20 μg/ml. For the nucleoproteins, which were prepared as gels in distilled water, dilution series were made with $NaNO_3$ solutions and a concentration giving an extinction at 260 mμ of about 0.300 chosen. The 'melting temperature' T_m was determined as usual from the plot of $E_t o/E_{25} o$ (at 260 mμ) against temperature, T_m being the temperature of half-maximal denaturation. The atomic extinction coefficient with respect to phosphorus, ε(P), was calculated according to the formula given by CHARGAFF and ZAMENHOF[10].

Results. Figure 1 shows an example of the denaturation curves obtained with the nucleoprotein, the 'crude' and the 'pure' DNA from consecutive purification steps of the same individual thymus material. The main distinctions in the behaviour of the different preparations are: (1) the higher the histone content, the higher the 'melting point' T_m, and (2) the higher the histone content, the smaller the increment of extinction at 260 mμ observed after heating to 95°C. This second characteristic is true for the comparison of the 'crude' and 'pure' DNA, but the intact nucleoproteins showed irregular behaviour, some preparations giving high values for $E_t o/E_{25} o$ around 90°C (up to 1.5). The low electrolyte concentration chosen for these experiments ($0.0025\,M$) gives sufficiently low T_m values for the 'pure' DNA preparations to demonstrate clearly the stabilizing effect of the additional histone retained in the 'crude' preparations. The Table shows these relationships in more detail for a number of experiments. In addition to the two points already mentioned, these figures demonstrate that the 'crude' preparations have greater ε(P) values than the 'pure', both in the native and in the denatured states. The increase in ε(P) during denaturation [$\Delta\varepsilon$(P)] is significantly higher in the 'pure' DNA (+ 2278,

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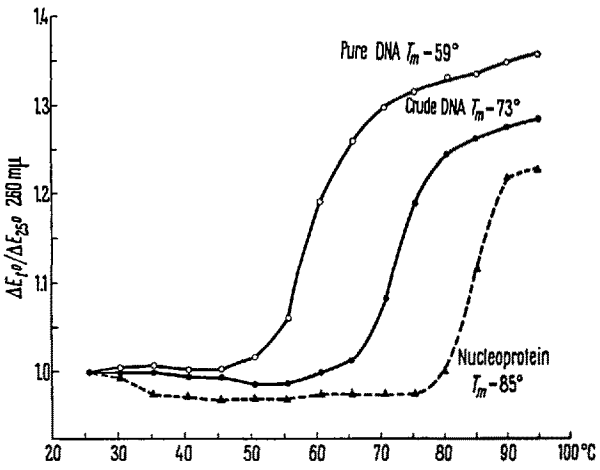
Atomic extinction coefficients ε(P) of 'crude' and 'pure' DNA preparations before and after thermal denaturation, 'melting point' T_m and residual histone content. The standard error is given

	'pure' DNA	'crude' DNA	Nucleoprotein
Residual histone (% of DNA)	0.43 ± 0.06 (14)	3.11 ± 0.26[a] (14)	—
ε(P) in native state	6725 ± 82 (10)	7457 ± 75[a] (11)	7538 ± 263 (6)
ε(P) in denatured state	8958 ± 127 (10)	9473 ± 71[a] (11)	—
$\Delta\varepsilon$(P) of individual experiments	+ 2278 ± 75 (10)	+ 2015 ± 87[b] (11)	—
$\Delta\varepsilon$(P) in % of ε(P) native	+ 33.8%	+ 27.0%	
T_m in 0.0025 M $NaNO_3$	58.5° ± 1.6° (8)	65.6° ± 1.9°[c] (9)	83.8° ± 1.4° (6)

[a] Difference crude-pure signif. at $p < 0.005$. [b] Difference crude-pure signif. at $p < 0.05$. [c] Difference crude-pure signif. at $p < 0.02$.

or 33.8%) than in the 'crude' DNA (+ 2015, 27.0%). Since the increase in UV-extinction at 260 mμ on heating a DNA solution is considered to be a sign of the breaking of the purine-pyrimidine hydrogen bonds holding the double helix together[11], the lower value for $\Delta\varepsilon$(P) shown by the DNA with the higher histone content would appear to indicate incomplete denaturation due to a stabilizing effect of the residual protein. The possible objection that the higher ε(P) of the unheated 'crude' DNA shows a partial denaturation, does not seem valid, since on further purification the 'pure' DNA with the expected ε(P) for undenatured DNA is obtained[6,12]. The considerable increase in T_m from the 'pure' to the 'crude' DNA and to the intact nucleoprotein also suggests a stabilizing effect of the histones on the DNA structure[13].



Thermal denaturation of 'pure' DNA (0.45% residual histone), 'crude' DNA (3.65% residual histone), and intact nucleoprotein from the same individual bovine thymus. Extinctions read at ambient temperatures, in 0.0025 M $NaNO_3$.

Zusammenfassung. Durch grösseren Gehalt an Resthiston wird die Stabilität von Desoxyribonucleinsäure gegenüber der thermischen Denaturierung in 0.0025 M $NaNO_3$ erhöht und die Zunahme des atomaren Extinktionskoeffizienten ε(P) während der Denaturierung verringert.

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Institut für experimentelle Gerontologie, Basel (Switzerland), November 18, 1964.

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Résultats de greffes cœlomiques d'ébauches gonadiques de Poulet de 4 jours

Le hasard est à l'origine du présent travail. Nous nous étions proposé d'étudier l'évolution du mésonéphros d'embryon de Poulet dans les conditions de la greffe cœlomique et avions choisi entre autres des embryons de 4 jours comme donneurs. Or, à ce stade, l'ébauche gonadique ne se détache pas encore très nettement du mésonéphros et il nous était arrivé, dans 3 cas sur douze, de la transplanter en tout ou en partie en même temps que le fragment de mésonéphros. Dans les 3 cas, elle s'était différenciée en un testicule apparemment normal au sein d'un hôte femelle qui, dans les 3 cas, était pratiquement dépourvu de canaux de Müller. Mais en soumettant les transplants à l'examen histologique, nous nous aperçûmes de la féminisation partielle de l'un d'eux (WENIGER et MALINOWSKA[1]). Nous avons voulu reproduire ces résultats.

Matériel et méthode. L'œuf de Poule de race Leghorn blanche (*Gallus gallus* L.) nous servit de matériel d'expérience.

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