Meanwhile, during lysis of the cells with toluene only one-quarter of the total protein present in cell-free extracts after disintegration of the cells by ultrasound was liberated into the extract.

The method of determining activity of restriction endonuclease Bam Hl in bacterial cells devised by the writers thus compares favorably with those described in the literature as regards simplicity, speed, small quantity of biomass required for analysis, good reproducibility, and dispensation with the need to obtain a cell-free extract, connected with ultrasonic disintegration of the cells and high-speed centrifugation. The method can probably be used also for preparative extraction of restriction endonucleases from cells, which would greatly simplify the procedure of their subsequent purification.

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POSSIBLE INDUCTION OF RECOMBINATION OF DNA FRAGMENTS FROM DIFFERENT TISSUES BY HYDROCORTISONE

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KEY WORDS: hydrocortisone; DNA; recombination.

According to data in the literature, after a single injection of nucleic acid precursor the label is intensively and rapidly incorporated into DNA of proliferating tissues. Later, during a steady reduction in the pool of radioactive precursor, the size of which is almost identical for all tissues, and, correspondingly, with a decrease in specific radioactivity of the DNA of tissues with a high mitotic index, the specific radioactivity of DNA of mitotically less active tissues rises [2]. It can be postulated on the basis of these findings that incorporation of label into DNA of tissues with a low mitotic index after a single injection of radioactive precursor can take place not only at the monomer level, but also by incorporation of entire fragments of DNA of mitotically active tissues. The DNA is evidently transported through the blood stream. Under these circumstances the main source of extracellular plasma DNA is considered to be normocytes, which have lost their Y nuclei and are thus converted into erythrocytes [9]. After injection of exogenous DNA into the blood stream of animals, it can be incorporated into the recipient's DNA [8]; this process, moreover, can take place also in the presence of hydroxyurea, which inhibits semiconservative DNA synthesis. The cells incorporate exogenous DNA, of both high and low molecular weight, but native DNA is incorporated much more efficiently than denatured DNA [12]. Not only homologous, but also heterologous DNA, injected into animals, can be incorporated into the recipient's genome, and this is accompanied by the appearance of the donor's genetic features [14]. We also know that recombination of mobile dispersed genes within the genome can take place in eukaryotes, in the same way as in prokaryotes [3]. Since incorporation of exogenous fragments into DNA

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TABLE 1. Trend of Changes in Radioactive Pool of DNA Precursor (in cps/ml cytosol) and Radioactivity of DNA (in cpm/mg DNA) after a Single Injection of [^{3}H]Thymidine

Tissue source	Experimental conditions	Time of injection of substances, h					
		2	12	24	48	96	168
DNA of bone marrow	Normal	88 911±5344	125 174±10 175	90 260±4 711	61 375±3 771	450 012±401	29 140±789
	Hydrocortisone	—	—	—	50 872±4 785	39 177±324	19 124±431
DNA of blood plasma	Normal	1 120±175	20 860±1 375	50 500±3 275	89 226±4 875	55 381±975	56 030±2 375
	Hydrocortisone	—	—	—	82 780±3 075	54 412±872	42 775±3 776
DNA of spleen	Normal	1 220±14	16 068±1 001	27 931±1 985	26 031±2 201	28 347±1 817	29 009±1 575
	Hydrocortisone	—	—	—	25 730±1 762	29 573±2 112	32 307±2 772
DNA of liver	Normal	1 250±24	1 514±101	3 144±207	5 175±278	7 035±572	12 134±2 017
	Hydrocortisone	—	—	—	6 582±378	10 877±691	19 591±1 875
	Hydrocortisone + hydroxyurea	—	—	—	7 135±629	11 382±907	20 518±973
	Hydrocortisone + nonradioactive thymidine	—	—	—	6 031±472	8 530±621	16 078±1 002
	Nonradioactive thymidine*	244±31	807±56	1 501±78	2 217±239	3 599±272	5 037±1 578
DNA of kidneys	Normal	1 109±17	1 031±89	1 503±127	2 519±217	3 078±207	4 093±575
	Hydrocortisone	—	—	—	3 124±173	3 710±211	5 071±272
Cytosol of liver	Normal	4 975±135	1 357±168	721±54	307±45	185±39	170±38
	Hydrocortisone	—	—	—	417±65	192±21	185±29
Cytosol of kidneys	Normal	4 832±201	1 419±106	613±41	325±32	199±31	191±12
	Hydrocortisone	—	—	—	374±22	225±17	227±24

Legend. Each value shown is arithmetic mean of three independent determinations; error indicated as standard deviation of arithmetic mean. * — Nonradioactive thymidine injected simultaneously with [^{3}H]thymidine; in all other versions all compounds used were injected 24 sec after injection of [^{3}H]thymidine.

of recipients' cells can take place only in the presence of pre-existing breaks in the polynucleotide chain, induction of additional breaks can evidently lead to more effective incorporation of DNA fragments from the blood plasma into DNA of the recipients' cells. The opinion also is held that gene induction, caused by hydrocortisone, is accompanied by the appearance of additional breaks in liver DNA, the mechanism of which is evidently based on excision demethylation of DNA [4], as is confirmed by activation of its postinduction reparative synthesis [1]. Demethylation of single-stranded regions of DNA [6] may lead to complete rupture of the chain. The lympholytic action of hydrocortisone also is known, and is expressed as activation of nucleases in these tissues, as a result of which enzymic degradation products of DNA from monomers to polynucleotide fragments with a molecular weight of up to $2.7 \cdot 10^6$ daltons are formed. DNA degradation products are found in both the blood and the urine [5].

The facts described above are evidence in support of the hypothesis that exchange of DNA fragments can take place between different tissues of the body. The investigation described below was carried out to test this hypothesis.

EXPERIMENTAL METHOD

Male Wistar rats weighing 190-210 g were used. The animals were given a single injection of [^{3}H]thymidine (55 Ci/mmole) in a dose of 250 μCi/100 g body weight in 2 ml of 0.9% NaCl, pH 7.4. Nonradioactive thymidine was injected in a dose of 25 mg/100 g body weight in 5 ml of 0.9% NaCl, pH 7.4. Hydrocortisone and hydroxyurea were injected in doses of 2 and 50 mg/100 g body weight respectively in 2 ml of 0.9% NaCl, pH 7.4. Control animals received corresponding volumes of the same carrier solution. The animals were decapitated. DNA was isolated after destruction of the material by grinding in liquid nitrogen by Marmur's method [11]. DNA-containing material was isolated from blood plasma after sedimentation of the

cells (5000g, 5 min) and addition of an equal volume of 1N NaOH by the method in [13]. The DNA content was determined by the reaction with diphenylamine as in [9]. Radioactivity of DNA was counted on "Millipore" membrane filters with pore size of 0.45 μ in a scintillation spectrometer (Intertechnique, France) for 10 min, using scintillator containing (in 1 liter) 700 ml toluene, 300 ml methylcellosolve, 4 g 2,5-diphenyloxazole, and 400 mg 1,4-di(5-phenyl-2-oxazolylbenzene). Radioactivity of the precursor pool in the cytosol of the test organs was determined after homogenization in a Dounce homogenizer, and later in a Potter-Elvehjem homogenizer (2000 rpm, 3 min), in 10 volumes of solution containing 0.15 M NaCl, 0.024 M EDTA, pH 7.4, followed by discarding of the residue precipitated on the addition of one-third of the volume of 1.5 M $HClO_4$. Radioactivity of 1 ml of the acid-soluble supernatant was counted after addition of 9 ml of dioxane scintillator, containing the same fluorophores as the toluene scintillator, and also 60 g naphthalene and 100 ml methanol per liter of dioxane.

EXPERIMENTAL RESULTS

The data in Table 1, on incorporation of radioactive label into DNA of different organs under normal conditions, agree with the results of the previous investigation [2]. Data obtained under experimental conditions show that injection of hydrocortisone led to more rapid removal of radioactive label from bone marrow DNA. This was accompanied by an increase in the DNA concentration in the blood plasma from 12.7 to 17.1 mg% 6 h after injection of the hormone and by a decrease in 8.9% mg 24 h after injection. This was followed by an increase in specific radioactivity of DNA in the spleen, liver, and kidneys compared with normal, against the background of reduced utilization of the pool of radioactive precursor. The results of a more detailed study of liver DNA show that, depending on the experimental conditions, hydrocortisone can either inhibit or potentiate incorporation of [^{3}H]thymidine into DNA. The first effect is seen if the content of the pool of radioactive precursor is high, the second if it is low. It can thus be postulated that in the second case intensively labeled blood plasma DNA is incorporated into liver DNA. However, the possibility likewise cannot be ruled out that [^{3}H]thymidine is incorporated into liver DNA in the course of reparative synthesis, activated by hydrocortisone and not inhibited, unlike replicative synthesis, by hydroxyurea [1]. However, to judge from the very weak effect of competitive inhibition of incorporation of [^{3}H]thymidine by nonradioactive thymidine, the suggestion that DNA fragments from blood plasma are incorporated into liver DNA still holds good. This process, as will be clear from the data described above, was independent of injection of hydroxyurea, just as in the case of injection of exogenous DNA into the blood stream [8].

To conclude, the suggestion that exchange of DNA fragments from different tissues is possible must be regarded as an hypothesis, and one of several possible interpretations of the data in the literature together with the writer's own observations. Inter-tissue recombination of DNA fragments, postulated on the basis of the results of the present investigation, is evidently a repair process, additional to recombinations within the genome [3], and possibly similar to postreplicative repair [7]. It can be tentatively suggested that activation of transcription by hydrocortisone and intensification of wear and tear of the DNA-template, produced in this way, and expressed as a disturbance of its structure, activate the hypothetical recombination repair, which perhaps incorporates inter-tissue exchange of DNA fragments.

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TRANSMEMBRANE POTENTIAL OF RAT LIVER MITOCHONDRIA IN HYPOTHYROIDISM

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Changes in oxidative phosphorylation of mitochondria caused by a deficiency of thyroid hormones in the body are now well known although the molecular mechanism of these changes still remains unexplained. The rate of oxidation of substrates in the presence of ADP has been shown to be depressed compared with normally [6]. It is also known that mitochondria, isolated from animals with hypothyroidism swell more slowly in media containing injury factors (oleate, phosphate anions, thyroxine) [10]. The reasons for these differences have not hitherto been analyzed. It was reported previously that the transmembrane potential (TMP) of liver mitochondria of hyperthyroid rats is higher than normal, whereas the system responsible for maintaining TMP in these mitochondria is less resistant to Ca^{++} ions than in the organelles of normal animals [2]. In the present investigation, by recording the value of TMP of mitochondria from the liver of hypothyroid animals, changes opposite to those observed when the thyroid hormone level was raised were observed: a decrease in TMP and an increase in resistance of the potential-maintaining system to Ca^{++} ions.

EXPERIMENTAL METHOD

Wistar rats weighing 180-200 g were used in the experiments. Mitochondria were isolated in 0.3 M sucrose with 10 mM Tris-HCl, pH 7.4, by the method in [5]. Experimental hypothyroidism was produced by thyroidectomy. The animals were used in the experiments 3-4 weeks after thyroidectomy. Animals undergoing a mock operation served as the control. The TMP level was judged from the intensity of quenching of fluorescence of a dis-C_3-(5) probe in a suspension of energized mitochondria (the $\Delta F/F$ parameter) [7]. The quantity of endogenous Ca^{++} in the mitochondria was determined by flame photometry on a Hitachi-207 atomic absorption spectrophotometer. The protein concentration was determined by the method of Lowry et al. [8].

EXPERIMENTAL RESULTS

TMP of energized mitochondria from the liver of hypothyroid rats was maintained longer than in preparations from control animals. This can be seen on curves of fluorescence of the probe in a suspension of mitochondria loaded with Ca^{++} ions (Fig. 1a, b). Additionally, as will be clear from this figure, more Ca^{++} had to be added to the preparations for development of a rapid decline of TMP than in the case of mitochondria of normal animals (Fig. 1c, d). Measurement of the parameter $\Delta F/F$ of the probe, the value of which is proportional to TMP [7], showed that this ratio is 83 ± 4% of normal (results of preparative isolations from eight normal and eight hypothyroid rats with four or five repetitions for each mitochondrial preparation). Consequently, if thyroid hormones are deficient in the body, the picture observed in the mitochondria is opposite to that revealed for organs of rats with hyperthyroidism [2]. In the latter case an increase in TMP was combined with a decrease of resistance of the mitochondria to the damaging action of Ca^{++} ions.

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