so ergibt sich für die Chinineffekte keine Korrelation, für die Kompensation der Phenylbutazonschädigung dagegen ist der Korrelationskoeffizient signifikant ($P < 0{,}01$). Die Regressionslinie ist in Figur 2 dargestellt.

Diskussion. Zusammenhänge zwischen der positiv inotropen Digitaliswirkung und intrazellulären Ionenveränderungen werden seit langem diskutiert (Literaturübersicht[11]). Die von einigen Autoren nachgewiesene Abgabe intrazellulären K^+ wurde neuerdings durch Befunde von KLAUS, KUSCHINSKY und LÜLLMANN[17] in Frage gestellt. Sie fanden durch therapeutische, positiv inotrop wirksame Digitoxigenin-Konzentrationen eine etwa 20%ige Abnahme des intrazellulären Na-Gehaltes und parallel dazu eine etwa 30%ige Abnahme des Ca-Gehaltes. Sie folgern aus ihren Befunden, dass eine Hemmung des Ionentransportes oder eine Abnahme des K- und Na-Gradienten bei der Herzmuskelzelle nicht die Ursache für den therapeutischen Effekt der Herzglykoside sein kann, sondern nur mit toxischen Glykosidwirkungen korreliert ist. Demgegenüber zeigen unsere Befunde, dass die Hemmung des aktiven Ionentransportes am Erythrocyten nicht nur mit den Toxizitätswerten, sondern auch mit den therapeutischen Effekten korreliert werden kann, und dass darüber hinaus diese Hemmwirkung die von uns nachgewiesenen Wirkungsunterschiede verschiedener Genine und Glykoside nach Schädigung zu erklären gestattet. Bei der Untersuchung des Einflusses verschiedener herzschädigender Verbindungen fanden wir[11] nach Phenylbutazon eine signifikante Zunahme des Na- und K-Gehaltes, die mit klinischen Berichten über eine verminderte Na-Ausscheidung im Urin übereinstimmt (Literatur bei RECHENBERG[18]). Nach Chinin dagegen kommt es zu einer Na-Abnahme bei gleichzeitiger K-Zunahme. Es ist daher verständlich, dass in unseren Versuchen die Hemmwirkung auf den Na-Transport nur mit den positiv inotropen Effekten nach Phenylbutazon-Schädigung korreliert war, nicht dagegen mit den Effekten nach Chinin. WILBRANDT[19,20] vertritt die Auffassung, dass ein gemeinsames Carriersystem für Na^+ und Ca^{++} existiert und die Verschiebungen beider Ionen gleichsinnig erfolgen.

Für die Erklärung der Wirkungsunterschiede zwischen den Digitaliskörpern dürfte ursächlich ein unterschiedlicher Effekt auf den Calciumgehalt wahrscheinlicher sein, der jedoch an den gleichsinnigen Na-Veränderungen leichter zu messen ist[21].

Summary. For therapeutic heart-equieffective concentrations of 15 genins, there is a correlation between the inhibition of the sodium transport in cold-stored red cells of man and the positive inotropic action on the isolated auricle preparation of the guinea pig, damaged by phenylbutazone.

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Acid-Soluble Purine and Pyrimidine Compounds of Mammalian Spermatozoa

Studies on the free nucleotides and related compounds of small molecular weight contained in spermatozoa have been so far confined to bull[1-5], ram[3,6], boar[6] and sea-urchin spermatozoa[7,8]. In this communication, we report the results of estimations, carried out as described earlier[4], of the intracellular pool of free nucleotides and related compounds in the semen of goat, buffalo and several indigenous breeds of bull.

Several workers have shown the presence of a sizable pool of adenosine triphosphate (ATP) in bull[1-3,5], ram[3,6], boar[6], and sea-urchin[7,8] spermatozoa, and of adenine di- and monophosphates (ADP and AMP, respectively) in bull[5] and sea-urchin[8] spermatozoa; the acid-soluble fraction of bull spermatozoa has also been reported to contain inosine and inosinic acid[5]. The presence of a pool of acid-soluble guanine, cytosine, uracil, or/and thymine derivatives in spermatozoa has, however, not been reported so far, except in the case of sea-urchin spermatozoa[8], which have been shown to contain almost half as much uridine triphosphate (which, according to the author, may have contained some guanosine triphosphate) as ATP. Since bull and buffalo spermatozoa have been shown to synthesize ribonucleic acid (RNA)[9], it seemed to be of interest to determine if the normal complement of free nucleotides is present in bovine spermatozoa. Mammalian spermatozoa will be expected to contain the triphosphates of cytosine and guanosine in addition to ATP[10], also in view of their ability to incorporate radioactive amino acids into their proteins[9,11-14]. In this communication, we also show that bull and buffalo spermatozoa contain significant amounts of acid-soluble derivatives of adenosine, guanosine and cytosine; uracil and thymine derivatives are not present, except perhaps in traces.

Materials and Methods. The semen was obtained from 3 bulls (Kerry, Deoni and Hanuman breeds), a buffalo

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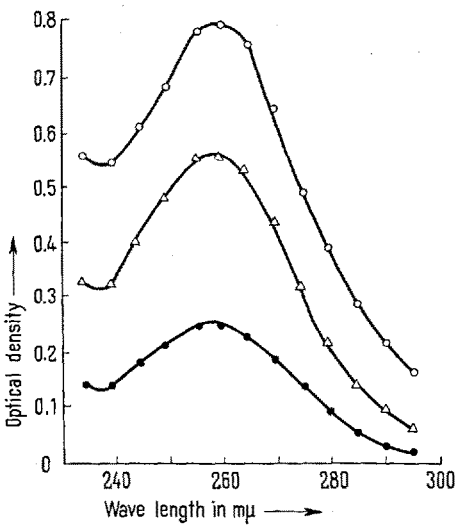
(Murrah breed) and a goat (Jamnapari breed) by the artificial vagina technique of WALTON[15]. All the above animals were of proved fertility. The semen was always used within 2 h of collection.

To obtain the acid-soluble fraction of spermatozoa, the semen (1–3 ml) was diluted with 4 times its vol of Krebs–Ringer original bicarbonate buffer[16] and centrifuged at room temperature for 15 min at 1000 *g*. The sedimented spermatozoa were washed once again as above and treated with 5 ml of PCA at 0°. After 5 min, the precipitate was removed by centrifugation in the cold and extracted once again as above with PCA. The ultraviolet absorption spectrum of the combined PCA extracts was determined in a 1 cm cell in a Beckman ultraviolet spectrophotometer, using a suitable PCA blank; the yield of the purine and pyrimidine derivatives was calculated from the value for the optical density at 260 mμ.

In some experiments, the PCA extract was neutralized with 0.3*N* KOH and the precipitated $KClO_4$ removed by centrifugation at 0°. The supernatant was evaporated to dryness under vacuum and hydrolysed with 72% PCA (0.05 ml) at 100° for 1 h. After removal of PCA as $KClO_4$ as described above, the nucleic acid bases in the hydrolysate were separated by paper chromatography following the method of SMELLIE et al[17]. The bases were eluted and estimated as described by BHARGAVA et al.[18].

Results and Discussion. As already reported for bull spermatozoa[4], the ultraviolet absorption spectra (Figure) of the acid-soluble fractions derived from the various animals studied in this investigation exhibited all the expected characteristics of the spectrum of a mixture of purine and pyrimidine derivatives which are normal constituents of the acid-soluble fraction of somatic cells. Very little variation was obtained in the above spectrum (absorbance ratios at 240/260, 250/260, 270/260, 280/260 and 290/260 mμ) from batch to batch of semen derived either from the same animal, or from different animals of the same species. The variation in this regard between the species studied was also insignificant.

The results of a series of estimations of the content of low mol weight purine and pyrimidine derivatives in goat, buffalo and bull spermatozoa, are given in Table I.



The ultraviolet spectra of the acid-soluble fractions from goat (•), buffalo (△), and bull (Hanuman breed, ○) spermatozoa. Each ml of the solution, of which the optical density values are plotted, was derived from 200×10^6 spermatozoa.

These results indicate that mammalian spermatozoa may, in general, have a large pool of free nucleotides and related compounds[4]. The size of this pool varied from 0.3–1.2% of the dry weight of spermatozoa for the 5 animals (belonging to 3 species) studied by us; in a previous study[4], this range was 0.4–1.1% of the dry weight of the cells, for several batches of semen from the same animal (a bull). These observations suggest that inter- and intra-species variations in the amount of acid-soluble 260 mμ-absorbing material present in mammalian spermatozoa, may not be more significant than the variation obtained in different batches of semen from the same individual animal.

Tab. I. A comparison of the content of acid-soluble purine and pyrimidine derivatives present in goat, buffalo and bull spermatozoa

Animal	Spermatozoa/ml semen ($\times 10^{-6}$)	Yield of the purine and pyrimidine derivatives in the acid-soluble fraction	
		pμ moles[a]/spermatozoon	% of the dry weight of spermatozoa[b]
Goat (Jamnapari)	4090	122	0.36
Buffalo (Murrah)	530	392	0.86
Bull (Deoni)	1980	157	0.33
Bull (Kerry)	1650	480	1.03
Bull (Hanuman)	585	543	1.15

[a] 1 pμ mole = 10^{-18} mole. The values given are based on an average molar extinction coefficient ($E^{260}_{1\,cm}$) of 10000 for purine andpyrimidine derivatives normally constituting the free nucleotide pool.

[b] The dry weight of goat, buffalo, and bull spermatozoa have been taken to be 11×10^{-12}, 18×10^{-12}, and 16.5×10^{-12} g respectively; the average mol weight of the purine and pyrimidine derivatives present in the acid-soluble fraction has been taken to be 350.

Tab. II. The relative abundance of purine and pyrimidine bases in the PCA-hydrolysate of acid-soluble fractions from buffalo and bull spermatozoa

Base	Moles/100 moles of the sum-total of the bases detected	
	Buffalo	Bull
Adenine	50.0	60.0
Guanine	16.0	22.3
Cytosine	34.0	17.7
Uracil[a]	0	0
Thymine[a]	0	0

[a] Under the conditions used, the sensitivity of the method was such that it should have been possible to detect these bases on the chromatogram if they were present in the spermatozoa in concentrations exceeding 10 pμ moles/cell, or 2 moles/100 moles of the sum-total of the bases present in the cell.

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LARDY et al.[2], MANN[3], and NEWTON and ROTHSCHILD[5] found 274, 314 and 129 ± 14 (range 79–161) pμ moles of ATP per cell, respectively, in freshly ejaculated bull spermatozoa[5]. NEWTON and ROTHSCHILD[5] also estimated the ADP, AMP, inosine and inosinic acid content of bull spermatozoa; their values for total adenosine and inosine derivatives (AMP + ADP + ATP + inosine + inosinic acid) ranged from 414 to 686 pμmoles/spermatozoon. The content of ATP alone in ram spermatozoa has been reported to be 164 pμ moles/cell[3,5]. A comparison of these values for adenosine (and inosine) derivatives in mammalian spermatozoa, with our values for the total acid-soluble purine and pyrimidine derivatives contained in these cells (Table 1), would suggest that the derivatives of other nucleic acid bases are not present in significant amounts. HULTIN[8], and NEWTON and ROTHSCHILD[5] had attempted a separation of the constituents of the acid-soluble fraction of spermatozoa by ion-exchange chromatography, and did not report the presence of any compounds other than the derivatives of adenine and hypoxanthine in the case of bull spermatozoa, and of adenine and uracil in the case of sea-urchin spermatozoa.

On paper chromatography of the hot PCA hydrolysate of the acid-soluble fraction of buffalo and bull spermatozoa, we, however, always obtained 3 distinct, clearly visible ultraviolet absorbing spots, which were shown to be due to adenine, guanine and cytosine by their Rf value and absorbance ratios at 250/260 and 280/260 mμ. The relative proportions of these 3 bases derived from the acid-soluble fractions of buffalo and bull spermatozoa in typical experiments, are given in Table II. Contrary to the results of earlier investigations[2,3,5,6], the acid-soluble cytosine and guanine derivatives put together were found to be present in these spermatozoa in nearly the same quantity as adenine derivatives.

Thymine and uracil were never detected on the paper chromatogram; their acid soluble derivatives can, therefore, be present in bull and buffalo spermatozoa only in traces. The virtual absence of thymine derivatives in the acid-soluble fraction of spermatozoa is understandable since these cells neither divide nor have any intracellular turnover of deoxyribonucleic acid[9]. The absence of uracil derivatives, is, however, surprising in view of the ability of mammalian spermatozoa to synthesise RNA[9].

Studies are currently in progress on the separation and quantitative estimation of each of the purine and pyrimidine derivatives present in the acid-soluble fraction of mammalian spermatozoa[10].

Zusammenfassung. Der Gehalt an säurelöslichen Purin- und Pyrimidinderivaten aus Stier-, Büffel- und Ziegenspermatozoen wurde bestimmt. Es wird gezeigt dass neben Adeninderivaten beträchtliche Mengen von Guanin- und Cytosinderivaten auch in diesen Zellen vorkommen. Uracil- und Thyminderivate waren nicht nachweisbar. Frühere Arbeiten über die freien Nukleotide aus Spermatozoa werden ebenfalls zusammengestellt.

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The Gonadotropic Action of Cecropia Extracts in Allatectomized American Cockroaches

Ovarian maturation and yolk deposition have been shown to be under the endocrine control of the corpus allatum in a variety of insects[1,2], including the American cockroach (*Periplaneta americana*)[3]. Although most of this work was accomplished by the extirpation and implantation of organs, recently WIGGLESWORTH[4] found that farnesol, a compound that shows juvenile hormone activity in certain insects[5], induces yolk formation when applied to the surface of the cuticle of decapitated adult females of *Rhodnius prolixus*. This latter observation was interpreted as providing further evidence for the probable identity of the yolk-forming (gonadotropic) hormone and the juvenile hormone. However, attempts to initiate ovarian development in allatectomized blow flies (*Calliphora erythrocephala*)[6] and Colorado potato beetles (*Leptinotarsa decemlineata*)[7] with insect extracts containing juvenile hormone have been unsuccessful. This paper reports the use of extracts of abdomens of the male cecropia moth (*Hyalophora cecropia*) to initiate ovarian development and yolk formation in allatectomized American cockroaches.

The cockroaches used in these tests were from a laboratory strain fed commercial dog food. Newly molted adult females were removed from the colony daily and held in containers with adult males. When the females had produced 1 to 3 ootheca, they were allatectomized and held under observation in individual containers for 14–21 days. This was generally found to be sufficient time for the complete regression and resorption of any oocytes undergoing development at the time of allatectomy. Allatectomized females that produced ootheca 7 or more days after the allatectomy were not used as test organisms.

To determine whether these allatectomized female roaches were capable of responding to gonadotropic hormone, 1 to 4 pairs of corpora allata were transplanted into each of 25 allatectomized females. Within 7 to 20 days, 23 of these roaches (92%) produced ootheca. With

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